# EIGHTH ANNUAL REPORT ON CACAO RESEARCH 1938

IMPERIAL MYCOLOGICAL
12 1939

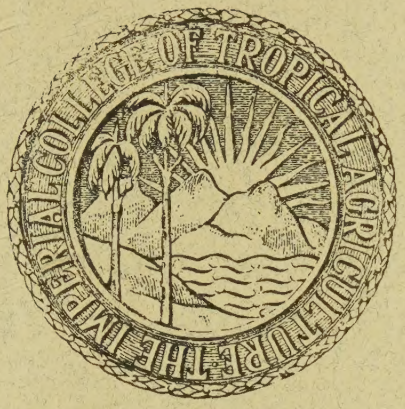


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PLATE 1.

Fig. 1.—I.C.S. 1, fan cutting, $2\frac{1}{2}$ years old.

Fig. 2.—I.C.S. 1, fan cutting, $2\frac{1}{2}$ years old.

PLATE 2.

Fig. 3.—I.C.S. 1, fan cutting, 3½ years old.

Fig. 4.—I.C.S. 4, fan cutting, 2½ years old.

# EIGHTH ANNUAL REPORT ON CACAO RESEARCH, 1938.

## A.—BOTANICAL SECTION.

## THE BOTANICAL PROGRAMME OF 1938.

By E. E. Cheesman.

Studies of self-incompatibility continue to engage the chief attention of the Cacao Research Botanist, and in particular the relationship between compatibility and yield has been studied.

Detailed observations on 600 young trees among seedling progenies at River Estate were mentioned last year. These have been continued, and Mr. Cope now presents conclusions from results covering a whole season. He has now proved statistically, what we have long suspected from general observations, that self-incompatible trees produce as a rule many more flowers than self-compatibles. This is perhaps a minor point, but worth demonstration. More important is his analysis of subsequent events, showing that the *percentage* of flowers which set is so much higher on the compatibles, that in spite of their lower intensity of flowering they set a higher *total number* of cherelles. Some of the initial advantage is lost through a higher percentage of shrivelling, but the compatible trees finally produce significantly more pods than the incompatibles.

For this particular population, Mr. Cope estimates that replacement of all the incompatible trees by compatibles might be expected to increase the yield by 24 per cent. The figure, as he is careful to point out, would probably be quite different for populations of different parentage or under different conditions. Nevertheless its magnitude is interesting. It is large enough to establish beyond doubt that compatibility is a factor of direct and practical importance in the economics of the Trinidad cacao industry. On the other hand, it is no very great figure when compared with the yield increases obtainable from certain manurial treatments, or with the difference in yields between trees on the selection list (whether self-compatible or not) and the average trees of the general population. We shall need much more information before we can estimate the losses due to incompatibility on estates in general, but at the present stage this 24 per cent. estimate places incompatibility in its right perspective, that is, as one important factor among many that are militating against high yields.

Among other additions to our knowledge during the last year, attention may be drawn to four items :

(1) As was expected, the seedling progenies of self-compatible trees by natural pollination include more compatibles than the corresponding progenies of self-incompatible trees. Of the six progenies studied, four came from self-incompatible parents and comprise approximately equal numbers of the two classes : the other two, from self-compatible parents, contain 61 per cent. and 76 per cent. of compatibles respectively. Another gap in our knowledge will be closed when progenies of self-compatibles by controlled self-pollination come into bearing. They have already been planted.

(2) Self-compatible trees vary among themselves in the actual percentage of flowers which they set from hand-pollination. Thus even among compatibles there are good, medium and relatively poor setters. The percentage also varies with the weather conditions at the time of pollination, but this fluctuation is not to be confused with the " conditional compatibility " that has been disproved ; it would appear to be rather due to destruction of pollen by rain or damp.

(3) Crosses between self-incompatible trees have still failed to reveal any cases of cross-compatibility, such as occur in some fruit-tree species.

(4) Preliminary studies of the mechanism of self-incompatibility indicate that it is *not* due, in cacao, to inhibition of pollen-tube growth in the style.

As in previous years, more work has been done than appears in the technical papers. The 576 trees of the first field experiment, planted at River Estate in 1937, are progressing satisfactorily. A second set of 576 was planted in 1938, and propagation started for a third set in 1939. In addition, a qualitative experiment was planted in which four contiguous monoclone blocks of self-incompatible trees have been surrounded with a border of the compatible I.C.S. 1. This is expected to yield, among other data, some information about the distance at which natural cross-pollination is effective.

We already have indications that the distance is considerable. In 1936 several hundreds of Criollo seedlings were inarched on Forastero roots for the Trinidad Department of Agriculture, and a solid block of them was planted by the courtesy of Mr. F. Agostini on San Juan Estate. These are now coming into bearing, and their pods show evidence of extensive cross-pollination from the surrounding Forastero trees. The trees in the middle of the block are some 300 yards from the nearest Forastero tree. Mr. Cope has commenced to record compatibilities and percentages of crossing throughout the block, and the results promise to be interesting.

We are also indebted to San Juan Estate, or rather to Mr. Agostini and to his able and enthusiastic manager, Mr. Robert de Verteuil, for a most encouraging demonstration of the value of the selections carried out between 1930 and 1935. A few spare cuttings of several types, presented to this estate in 1936, were included in their replanting programme, and in the current season, at an age of 2½ years, most of the young trees have been laden with pods. Cuttings of I.C.S. 1 are particularly impressive, and the photographs presented in Plates 1 and 2 speak for themselves. Yield data will be available next year, but there is already no doubt that several of these 2½ year trees will return more than 2 lb. of dry cacao each.

In view of results like that, the possibility of using vegetatively propagated planting material on a commercial scale is beginning to attract attention. We accordingly propose, during the current year, to keep careful records of our costs in the propagation of cuttings. It would have been useless to do so earlier, on account of complications introduced by experiments and by shortage of some classes of material. Now that we have abundance of nursery material and a fairly straightforward propagation programme it becomes feasible. In this connection it may be of interest to add that the present output of our propagators is between 250 and 300 rooted cuttings a month, and we expect to raise it a little higher without additional equipment. I again have pleasure in acknowledging the good work of Mr. Spencer in charge of propagation.

It is also a pleasure to record that our co-operation with the Trinidad Department of Agriculture continues to be as close as our research functions require or permit, and in particular to thank the Director and officers of that Department for their very generous attitude towards our plantings and other interests on River Estate.

## SOME FACTORS CONTROLLING THE YIELD OF YOUNG CACAO—II.

By F. W. Cope.

In the previous Report (3) a preliminary paper was presented to illustrate the effects of self-compatibility and self-incompatibility upon the several factors which determine yield, flowering, setting and cherelle maturation, in cacao. The results given in this second paper cover a whole season, and in every way corroborate the early findings.

It will be recalled that the observations were made on two acres of seedling cacao trees, arranged in six plots; each plot of 99 trees was planted, in 1933, with seed from one of six selected female parent trees. Fortnightly counts of flowers, young cherelles, wilted cherelles, and mature pods were made on each tree. Hand self-pollinations were made at the same time to give an indication of each tree's ability or inability to set with its own pollen.

Each plot carried a manurial experiment, four sub-plots, each of twelve trees, receiving different treatments. An attempt has been made to assess the relative effects of the two elements involved, phosphorus and potassium, in the various stages, from flowering to mature, harvested pod.

### Experimental Results.

Comparisons of behaviour of trees, classed as self-compatible and as self-incompatible, with respect to setting of cherelles, wilting of cherelles, flower production and pod yields, are presented in Tables I to III (*b*).

As explained in the preliminary paper (3), the figures quoted for flower production are obtained from the fortnightly counts by multiplying by ten, this factor allowing for the small proportion of flowers retained on the trees for two days after opening.

The strongly contrasted behaviour of the two trees, one self-compatible and one self-incompatible, is very apparent. Both trees are from the control sub-plot of plot 1A, and both produced approximately the same numbers of flowers over the whole course of the year.

The two right hand columns of each table indicate the trees' reactions to hand self-pollinations. The self-compatible 1A 71 set a proportion of flowers self-pollinated on almost every occasion, while the self-incompatible 1A 86 consistently set nothing from self-pollinations.

The inability of the self-incompatible tree to set with its own pollen is reflected in its low natural set of cherelles; only eleven cherelles resulted from a little over 10,000 flowers, a remarkably inefficient achievement. On the other hand, the self-compatible tree set a very much greater proportion of cherelles from its flowers. It should be borne in mind that the number quoted (135) for the total of new cherelles includes those cherelles which resulted from hand self-pollinations and which reached the age of about six weeks. The inclusion of selfed settings amongst the naturally set cherelles is regretted; but allowance can

**Table I.**

YEAR'S RECORD FOR A SELF-COMPATIBLE AND A SELF-INCOMPATIBLE TREE, BOTH BEING FROM THE CONTROL SUB-PLOT OF PLOT 1A.

| | Self-compatible Tree—1A 71. | | | | | | Self-incompatible Tree—1A 86. | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date. | No. New Cherelles. | No. Wilted Cherelles. | No. Flowers. | No. Pods Harvested. | No. Flowers Hand-Pollinated. | No. on after 14 days. | No. New Cherelles. | No. Wilted Cherelles. | No. Flowers. | No. Pods Harvested. | No. Flowers Hand-Pollinated. | No. on after 14 days. |
| 20. 7.37 | 1 | ... | 2180 | | 20 | 8 | | | 860 | | 15 | 0 |
| 4. 8.37 | 10 | ... | 1260 | | | | | | 180 | | | |
| 19. 8.37 | 20 | 1 | 160 | | | | | | 200 | | | |
| 7. 9.37 | 3 | 8 | 470 | | | | | | 430 | | | |
| 19. 9.37 | 4 | 1 | 1030 | | | | | | 180 | | | |
| 4.10.37 | 4 | 17 | 690 | | 15 | 6 | | | 300 | | 7 | 0 |
| 18.10.37 | ... | 9 | 240 | | | | 1 | | 640 | | | |
| 1.11.37 | 1 | ... | 140 | | | | | 1 | 300 | | | |
| 15.11.37 | 5 | 1 | 480 | | 15 | 5 | | | 170 | | 2 | 0 |
| 29.11.37 | ... | 1 | 140 | 2 | 10 | 7 | | | 120 | | 10 | 0 |
| 13.12.37 | 1 | ... | 420 | 1 | | | | | 570 | | | |
| 27.12.37 | 7 | ... | 430 | 1 | | | | | 260 | | | |
| 17. 1.38 | 30 | ... | 330 | | | | | | 20 | | | |
| 29. 1.38 | 12 | ... | 370 | | 10 | 6 | | | 60 | | | |
| 14. 2.38 | 9 | 2 | 130 | 1 | 9 | 7 | | | 20 | | | |
| 28. 2.38 | 16 | 15 | 90 | 1 | | | 6 | | 150 | | | |
| 14. 3.38 | 3 | 15 | 20 | 1 | 6 | 2 | | | 50 | | | |
| 28. 3.38 | 3 | 9 | 150 | 1+2* | 10 | 0 | | 6 | 20 | | | |
| 9. 4.38 | ... | 6 | 70 | | | | | | 30 | | | |
| 23. 4.38 | ... | 1 | 130 | 2+3* | 5 | 0 | | | 100 | | | |
| 7. 5.38 | ... | 1 | 60 | 3+5* | | | 1 | | 450 | | | |
| 21. 5.38 | ... | ... | 100 | 3+8* | | | | | 1150 | | | |
| 6. 6.38 | 1 | 1 | 160 | 5 | 8 | 5 | 1 | | 620 | | | |
| 20. 6.38 | ... | ... | 180 | 2 | | | | | 380 | | | |
| 4. 7.38 | 5 | 1 | 150 | | 9 | 4 | | | 430 | | | |
| 18. 7.38 | ... | 6 | 120 | | | | 2 | | 2350 | | | |
| Totals ... | 135 | 95 | 9700 | 23+18* | 117 | 50 | 11 | 7 | 10040 | 0 | 34 | 0 |

* For explanation, see text.

readily be made, and the estimated numbers of naturally set cherelles appear in Table II. For 1A 71, the number of cherelles set naturally was 89, over eight times as many as the self-incompatible 1A 86, and therefore still very significantly different.

Direct comparison may be made between the numbers of mature pods taken from the two trees. Pods resulting from hand self-pollinations were recorded separately and appear in the appropriate column of Table I marked with an asterisk. Tree 1A 71 produced 23 pods from natural pollinations, while 1A 86 bore no pods at all.

The superiority of the self-compatible tree with respect to cherelle and pod production over its self-incompatible neighbour is obvious from Tables I and II.

**Table II.**

YEAR'S RECORD FOR THE SELF-COMPATIBLE AND SELF-INCOMPATIBLE MEMBERS OF PLOT 1A.

| Self-compatible Trees. | | | | | | | Self-incompatible Trees. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Tree No. | Total Cherelle Set. | Natural Cherelle Set. | Wilted Cherelles. | Flowers. | Total Pods. | Naturally Set Pods. | Tree No. | Natural Cherelle Set. | Wilted Cherelles. | Flowers. | Pods. |
| Control Sub-plot. | | | | | | | Control Sub-plot. | | | | |
| 1A 63 | 84 | 50 | 54 | 2960 | 15 | 8 | 1A 62 | 18 | 12 | 15590 | 4 |
| 65 | 107 | 67 | 68 | 4630 | 14 | 6 | 64 | 9 | 3 | 4630 | 4 |
| 68 | 24 | 15 | 17 | 3550 | 7 | 5 | 69 | 17 | 8 | 2790 | 9 |
| 70 | 57 | 40 | 25 | 7120 | 16 | 12 | 85 | 31 | 6 | 8770 | 21 |
| 71 | 135 | 89 | 95 | 9700 | 41 | 23 | 86 | 11 | 7 | 10040 | 0 |
| 84 | 1 | 0 | 1 | 530 | 0 | 0 | | | | | |
| 87 | 25 | 15 | 19 | 3740 | 1 | 1 | | | | | |
| P Sub-plot. | | | | | | | P Sub-plot. | | | | |
| 1A 57 | 146 | 96 | 90 | 7850 | 49 | 31 | 1A 60 | 12 | 1 | 5210 | 3 |
| 58 | 104 | 69 | 66 | 7050 | 17 | 8 | 76 | 38 | 26 | 7830 | 13 |
| 59 | 3 | 0 | 2 | 2540 | 1 | 0 | 79 | 1 | 0 | 910 | 0 |
| 73 | 71 | 68 | 39 | 12610 | 26 | 23 | 80 | 1 | 1 | 6280 | 0 |
| 74 | 36 | 10 | 23 | 4930 | 12 | 4 | 81 | 1 | 1 | 2290 | 0 |
| 75 | 16 | 5 | 14 | 3280 | 0 | 0 | 82 | 1 | 1 | 2660 | 0 |
| K Sub-plot. | | | | | | | K Sub-plot. | | | | |
| 1A 26 | 89 | 71 | 26 | 2140 | 61 | 50 | 1A 18 | 41 | 17 | 24420 | 16 |
| 41 | 8 | 0 | 5 | 3710 | 0 | 0 | 20 | 1 | 0 | 6240 | 0 |
| 43 | 0 | 0 | 0 | 310 | 0 | 0 | 21 | 26 | 8 | 10360 | 14 |
| | | | | | | | 24 | 33 | 22 | 6650 | 10 |
| | | | | | | | 25 | 0 | 0 | 1040 | 0 |
| | | | | | | | 27 | 56 | 28 | 14650 | 21 |
| | | | | | | | 42 | 68 | 20 | 17270 | 37 |
| PK Sub-plot. | | | | | | | PK Sub-plot. | | | | |
| 1A 13 | 82 | 60 | 65 | 4000 | 10 | 8 | 1A 16 | 43 | 9 | 21910 | 30 |
| 14 | 17 | 11 | 12 | 2690 | 3 | 2 | 30 | 7 | 6 | 5160 | 1 |
| 15 | 29 | 22 | 17 | 2530 | 7 | 7 | 37 | 115 | 80 | 21060 | 29 |
| 29 | 34 | 24 | 14 | 2760 | 17 | 14 | | | | | |
| 31 | 6 | 2 | 6 | 1690 | 0 | 0 | | | | | |
| 32 | 283 | 228 | 194 | 18480 | 60 | 55 | | | | | |
| 35 | 70 | 48 | 49 | 3410 | 13 | 8 | | | | | |
| 36 | 63 | 40 | 49 | 5200 | 4 | 3 | | | | | |
| 38 | 14 | 6 | 13 | 1620 | 1 | 0 | | | | | |

### Statistical Treatment of Results.

The significances of differences in behaviour of the self-compatible and self-incompatible members of Plot 1A, with respect to the various stages of crop production, are statistically estimated below by Student's method (1). No attempt is made here to introduce manurial effects, the four sub-plots being treated as a whole.

(1) Flowering Intensity, Per Tree. Plot 1A.

| | Mean. | Sum of Squares. | Degrees of Freedom. | t | p |
|---|---|---|---|---|---|
| Self-compatible trees ... ... | 4960 | 337,780,100 | 23 | 2.655 | 0.01 |
| Self-incompatible trees ... | 9408 | 1,017,147,000 | 20 | | |

There is therefore a very significant difference in the intensity of flowering between the two classes of tree. Self-incompatible trees tend to produce more flowers than the self-compatible trees.

(2) Number of Cherelles Set Per 10,000 Flowers Borne, Per Tree. Plot 1A.

| | Mean. | Sum of Squares. | Degrees of Freedom. | t | p |
|---|---|---|---|---|---|
| Self-compatible trees ... | 84.3 | 120,746 | 22 | 3.600 | < 0.01 |
| Self-incompatible trees ... | 23.3 | 7,379 | 20 | | |

The very high significance of the difference of the means for the whole of the four sub-plots is corroboration of the differences noted in Table I, where two single trees are compared. Taking the plot as a whole, the self-compatible members set about three and a half times as many cherelles as the self-incompatible trees, equal numbers of flowers being considered. In other words, seven flowers on the self-compatible tree receive suitable pollen while the self-incompatible tree has only two flowers suitably pollinated in the same time. A self-compatible tree will set with its own pollen, and with pollen from any other cacao tree, self-compatible or self-incompatible, while the self-incompatible tree will set only with pollen from a self-compatible tree, thereby rendering itself entirely dependent upon the transference of pollen from a neighbouring self-compatible tree before any setting of cherelles can ensue. The importance of self-compatible pollinators in the mixed cacao population cannot therefore be over-emphasised.

(3) Actual Set of Cherelles, Per Tree. Plot 1A.

| | Mean. | Sum of Squares. | Degrees of Freedom. | t | p |
|---|---|---|---|---|---|
| Self-compatible trees ... ... | 41.4 | 58,504 | 24 | 1.327 | 0.2 |
| Self-incompatible trees ... | 25.2 | 16,322 | 20 | | |

Although the self-compatible tree still possesses an advantage over the self-incompatible tree with respect to the average actual cherelle set, the difference is not statistically significant. This is due to the greater numbers of flowers borne by the self-incompatible trees, offsetting somewhat the superior ease of setting to be found amongst the self-compatibles.

(4) Percentage Wilt of Cherelles, Per Tree. Plot 1A.

| | Mean. | Sum of Squares. | Degrees of Freedom. | t | p |
|---|---|---|---|---|---|
| Self-compatible trees ... ... | 67.1 | 5,535 | 21 | 3.319 | > 0.01 |
| Self-incompatible trees ... | 46.8 | 7,266 | 14 | | |

In estimating the means given above, all trees which produced less than six cherelles were omitted from the calculations. The highly significant value of $t$ indicates a very marked tendency amongst the self-compatible trees towards a degree of wilting greater than amongst the self-incompatibles. The figures may be viewed in a different way: of every 100 cherelles set on a self-compatible and a self-incompatible tree respectively, there will be left approximately only 33 on the self-compatible and 54 on the self-incompatible. There will be, therefore, a distinct advantage in favour of the self-incompatible tree, tending to reduce still further the preliminary lead in the matter of cherelle set which the self-compatibles possess.

(5) Actual Pod Yields, Per Tree. Plot 1A.

| | Mean. | Sum of Squares. | Degrees of Freedom. | t | p |
|---|---|---|---|---|---|
| Self-compatible trees ... ... | 10.7 | 5,407 | 24 | 0.1768 | 0.85 |
| Self-incompatible trees ... | 10.0 | 2,696 | 20 | | |

It is not surprising, in view of the militating effects of lower flowering intensity and higher cherelle mortality, to find that when the end product is considered—the mature pod—there is little advantage in favour of the self-compatible tree over the self-incompatible tree. Whilst this is true for the plot under consideration, reference to Table III (*a*), where the figures for the six plots are listed, shows that the superiority of the self-compatible tree at the fruit setting stage is in most cases maintained throughout the succeeding stages, resulting in better average pod yields from the self-compatibles compared with the self-incompatibles.

(6) Number of Pods Matured from 10,000 Flowers, Per Tree. Plot 1A.

| | Mean. | Sum of Squares. | Degrees of Freedom. | t | p |
|---|---|---|---|---|---|
| Self-compatible trees ... ... | 22.5 | 50,740 | 24 | 1.317 | 0.2 |
| Self-incompatible trees ... | 9.1 | 1,781 | 20 | | |

The superiority of the self-compatible tree over the self-incompatible tree is more apparent when the effects of flowers are eliminated, by expressing the pod production as so many per 10,000 flowers. Even on this basis, the difference can be ascribed no statistical significance. Reference to Table III (*b*) indicates that on four of the six plots, this difference is actually significant.

**Table III (a).**

Comparisons of Behaviour for the Six Plots, 1A-6C.—Tree Averages Quoted.

| Plot. | Flowers | | Cherelles Set | | Cherelles per 10,000 Flowers | | % Wilt | | Pods Harvested | | Pods per 10,000 Flowers | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Com-patible | In-com-patible | Com-patible | In-com-patible | Com-patible | In-com-patible | Com-patible | In-com-patible | Com-patible | In-com-patible | Com-patible | In-com-patible |
| 1A ... ... | 4960 | 9408 | 41.4 | 25.2 | 84.3 | 23.3 | 67.1 | 46.8 | 10.7 | 10.0 | 22.5 | 9.1 |
| 2D ... ... | 2429 | 3450 | 37.7 | 11.4 | 166.9 | 36.8 | 68.2 | 43.7 | 7.8 | 4.3 | 33.4 | 11.4 |
| 3E ... ... | 4755 | 5706 | 43.2 | 17.4 | 90.8 | 29.2 | 59.7 | 48.1 | 10.4 | 6.2 | 26.2 | 10.2 |
| 4B ... ... | 3044 | 3598 | 36.2 | 11.3 | 138.7 | 30.0 | 68.6 | 51.6 | 5.8 | 3.5 | 36.4 | 7.5 |
| 5F ... ... | 2426 | 6514 | 21.5 | 7.4 | 92.5 | 9.0 | 73.0 | 55.5 | 3.5 | 1.1 | 12.5 | 2.1 |
| 6C ... ... | 2531 | 2568 | 39.7 | 5.5 | 138.0 | 24.1 | 65.1 | 37.5 | 7.0 | 1.1 | 23.1 | 5.7 |
| Averages ... | 3358 | 5207 | 36.6 | 13.0 | 118.5 | 25.4 | 67.0 | 47.2 | 7.5 | 4.4 | 25.7 | 7.7 |

**Table III (b).**

SIGNIFICANCES OF VALUES OF 't' FOR THE ABOVE PAIRS OF RESULTS.

| Plot | Flowers | Cherelles Set | Cherelles per 10,000 flowers | % wilt | Pods Harvested | Pods per 10,000 flowers |
|---|---|---|---|---|---|---|
| 1A | 0.01 | 0.20 | <0.01 | <0.01 | 0.85 | 0.20 |
| 2D | 0.15 | 0.02 | <0.01 | <0.01 | 0.25 | 0.05 |
| 3E | 0.40 | <0.01 | <0.01 | 0.10 | 0.05 | 0.03 |
| 4B | 0.60 | <0.01 | <0.01 | 0.02 | 0.10 | 0.05 |
| 5F | <0.01 | 0.10 | <0.01 | 0.05 | 0.25 | 0.10 |
| 6C | None | <0.01 | <0.01 | <0.01 | 0.01 | 0.02 |

Values of 't' less than 0.05 considered significant.

The differences in behaviour between self-compatible and self-incompatible trees can readily be discerned from Tables III (*a*) and III (*b*). They may be summarised as follows:

(*a*) On all plots the self-compatibles produce less flowers than the self-incompatibles, although the significance of these differences is not always of a high order.

(*b*) On all plots the self-compatibles set more cherelles per tree than the self-incompatibles. The significance of these differences is often of a high order.

(*c*) On all plots the self-compatibles set a far higher proportion of flowers as young cherelles than do the self-incompatibles. The difference on every plot is consistently highly significant; indeed, it will be seen, on reference to Table III (*b*), that this difference in behaviour is the most strongly marked of all the features studied.

(*d*) On all plots the self-compatible element loses more of the cherelles through wilt than does the self-incompatible element. The significances ascribable to these differences are of a very high order.

(*e*) On all plots the self-compatibles produce more harvested pods than the self-incompatibles, though only on two of the six plots is this difference statistically significant.

(*f*) On all plots the self-compatibles produce more pods from a given number of flowers than do the self-incompatibles from the same number of flowers. Four of the six plots show a significant value of 't' in this respect.

## The Effect of Manures.

In this section the effects of manures upon the various stages of fruit production are introduced, together with their interactions with self-compatibility and self-incompatibility. The results quoted in the previous part of the paper apply to the mean tree values for all four sub-plots taken together and are sub-divided solely on the basis of compatibility and incompatibility. In this second part, the figures given in the tables are the mean values for individual sub-plots: these figures are treated by Fisher's (1) method of analysis of variance.

(1) Flowering Intensity, Plots 1A-6C. Tree Averages Quoted.

| Plot. | Control sub-plot | | P sub-plot | | K sub-plot | | PK sub-plot | |
|---|---|---|---|---|---|---|---|---|
| | Compatible. | Incompatible. | Compatible. | Incompatible. | Compatible. | Incompatible. | Compatible. | Incompatible. |
| 1A ... ... ... | 4610 | 8360 | 6380 | 4190 | 2050 | 11520 | 4710 | 16140 |
| 2D ... ... ... | 1890 | 620 | 2760 | 2130 | 2080 | 5930 | 2750 | 4080 |
| 3E ... ... .... | 4410 | 3900 | 4920 | 7860 | 6780 | 3290 | 3470 | 8640 |
| 4B ... ... ... | 6430 | 1190 | 2440 | 2030 | 2100 | 5070 | 3870 | 7280 |
| 5F ... ... ... | 2150 | 11680 | 2390 | 4000 | 3610 | 7410 | 1710 | 2950 |
| 6C ... ... ... | 2120 | 5150 | 2730 | 3770 | 3250 | 2350 | 2180 | 2110 |
| Totals ... ... | 21610 | 30900 | 21620 | 23980 | 19870 | 35570 | 18690 | 41200 |

| Variance due to | Degrees of Freedom. | Sum of Squares. | Mean Square. | $\frac{V_1}{V_2}$ | $\frac{1}{2}\log_e \frac{V_1}{V_2}$ | P |
|---|---|---|---|---|---|---|
| Total ... ... ... | 47 | 435,062,300 | | | | |
| Whole sub-plots ... ... | 23 | 197,991,900 | 8,608,300 | 1.6848 | 0.2608 | |
| Plots ... ... ... | 5 | 113,359,200 | 22,671,800 | 4.4373 | 0.7450 | 0.01 |
| K ... ... ... | 1 | 6,177,700 | 6,177,700 | 1.2090 | 0.0948 | |
| P ... ... ... | 1 | 126,100 | 126,100 | — | | |
| PK ... ... ... | 1 | 1,688,500 | 1,688,500 | — | | |
| Error (*a*) ... ... | 15 | 76,640,400 | 5,109,400 | | | |
| Compatibles *v.* incompatibles | 1 | 51,792,100 | 51,792,100 | 6.2533 | 0.9166 | Between 0.01 & 0.05 |
| Compatibility/manures ... | 3 | 19,629,900 | 6,543,300 | — | | |
| Error (*b*) ... ... | 20 | 165,648,400 | 8,282,400 | | | |

The significant plot effect may be due either to position or to genetical factors controlling flowering intensity. The latter seems more likely than the former, since the lowest flowering plot, 2D, is next to the highest flowering plot, 1A.

Manurial effects are not significant, but quite obviously potassium exerts a far greater influence upon flowering than does phosphorus. In view of the naturally deficient potash status of River Estate soils, this greater variance in the case of potassium is to be expected.

In confirmation of the results obtained in the first part of this paper, the difference between self-compatibles and self-incompatibles is statistically significant. There is no statistically significant differential effect of manures upon either class of tree; both the compatibles and incompatibles, however, are not quite equally influenced by manures, since the compatible manurial totals tend to decrease, whilst the incompatible totals tend to increase. The difference between compatible and incompatible totals on the control plot is just half that of the PK plot.

(2) Cherelles Set per 10,000 Flowers Borne.

| Plot. | Control sub-plot | | P sub-plot | | K sub-plot | | PK sub-plot | |
|---|---|---|---|---|---|---|---|---|
| | Compatible. | Incompatible. | Compatible. | Incompatible. | Compatible. | Incompatible. | Compatible. | Incompatible. |
| 1A ... ... ... | 90.6 | 27.6 | 51.6 | 15.4 | 165.9 | 24.4 | 83 8 | 29.3 |
| 2D ... ... ... | 264.5 | 48.4 | 106.7 | 25.2 | 139.2 | 23.4 | 234.6 | 41.7 |
| 3E ... ... ... | 86.9 | 15.9 | 93.4 | 35.7 | 78.0 | 31.5 | 101.3 | 29.2 |
| 4B ... ... ... | 65.3 | 20.5 | 144.4 | 66.5 | 129.8 | 12.1 | 149.7 | 30.3 |
| 5F ... ... ... | 107.1 | 8.4 | 89.7 | 31.8 | 67.9 | 14.3 | 98.4 | 5.3 |
| 6C ... ... ... | 138.7 | 24.1 | 169.4 | 37.5 | 101.7 | 24.1 | 80.1 | 13.1 |
| Totals ... ... | 753.1 | 144.9 | 655.2 | 212.1 | 682.5 | 129.8 | 747.9 | 148.9 |

| Variance due to | Degrees of Freedom. | Sum of Squares. | Mean Square. | $\frac{V_1}{V_2}$ | $\frac{1}{2}\log_e \frac{V_1}{V_2}$ | P |
|---|---|---|---|---|---|---|
| Total ... ... ... | 47 | 166,217.3 | | | | |
| Whole sub-plots ... ... | 23 | 41,320.3 | 1,796.5 | | | |
| Plots ... ... ... | 5 | 17,314.2 | 3,462.8 | 2.2006 | 0.3944 | Not sig. |
| K ... ... ... | 1 | 65.8 | 65.8 | | | |
| P ... ... ... | 1 | 60.3 | 60.3 | | | |
| PK ... ... ... | 1 | 276.4 | 276.4 | | | |
| Error (*a*) ... ... | 15 | 23,603.6 | 1,573.6 | | | |
| Compatibles *v.* incompatibles | 1 | 101,108.5 | 101,108.5 | 90.461 | 2.2525 | < 0.01 |
| Compatibility/manures ... | 3 | 1,435.1 | 478.4 | | | |
| Error (*b*) ... ... | 20 | 22,353.4 | 1,117.7 | | | |

In the above table, the only significant feature is the difference in behaviour between the compatibles and incompatibles.

From the almost negligible variance contributed by the manures, it may readily be concluded that neither phosphorus nor potassium exerts any effect upon the proportion of flowers ultimately setting to cherelles of six weeks of age. Furthermore, the insignificance of the interaction between compatibility and manures indicates that manurial effects, if any, do not discriminate between self-compatibles and self-incompatibles.

Actual Set of Cherelles Per Tree.

| Plot. | Control sub-plot | | P sub-plot | | K sub-plot | | PK sub-plot | |
|---|---|---|---|---|---|---|---|---|
| | Compatible. | Incompatible. | Compatible. | Incompatible. | Compatible. | Incompatible. | Compatible. | Incompatible. |
| 1A ... ... ... | 39.4 | 17.2 | 41.3 | 9.0 | 35.5 | 32.1 | 49.0 | 55.0 |
| 2D ... ... ... | 54.5 | 3.0 | 17.6 | 7.5 | 28.0 | 14.6 | 55.1 | 17.0 |
| 3E ... ... ... | 44.0 | 6.3 | 40.8 | 26.0 | 59.8 | 11.3 | 33.7 | 27.3 |
| 4B ... ... ... | 42.0 | 3.3 | 45.3 | 17.7 | 20.7 | 7.0 | 43.8 | 22.3 |
| 5F ... ... ... | 14.2 | 14.0 | 23.2 | 13.8 | 38.2 | 8.3 | 23.0 | 3.0 |
| 6C ... ... ... | 49.8 | 2.0 | 51.3 | 10.4 | 34.3 | 5.7 | 19.0 | 1.7 |
| Totals ... ... | 243.9 | 45.8 | 219.5 | 84.4 | 216.5 | 79.0 | 223.6 | 126.3 |

| Variance due to | Degrees of Freedom. | Sum of Squares. | Mean Square. | $\frac{V_1}{V_2}$ | $\frac{1}{2}\log_e\frac{V_1}{V_2}$ | P |
|---|---|---|---|---|---|---|
| Total ... ... ... | 47 | 13,960.5 | | | | |
| Whole sub-plots ... ... | 23 | 4,276.4 | 185.9 | | | |
| Plots ... ... ... | 5 | 1,611.0 | 322.2 | | | |
| K ... ... ... | 1 | 55.9 | 55.9 | | | |
| P ... ... ... | 1 | 98.0 | 98.0 | | | |
| PK ... ... ... | 1 | 33.7 | 33.7 | | | |
| Error (*a*) ... ... | 15 | 2,477.8 | 165.2 | | | |
| Compatibles *v.* incompatibles | 1 | 6,721.3 | 6,721.3 | 53.175 | 1.9868 | < 0.01 |
| Compatibility/manures ... | 3 | 434.5 | 144.8 | | | |
| Error (*b*) ... ... | 20 | 2,528.3 | 126.4 | | | |

The only striking significant difference lies in that between self-compatibles and self-incompatibles.

It is of interest to note, however, that manures, while still without statistically significant effect, do show some tendency towards increasing the numbers of cherelles set on the self-incompatible trees, the numbers for the self-compatibles remaining more or less uniform on all four sub-plots. Since this effect is not observable in the cherelles/10,000 flowers table, it must be assumed that the slight discriminatory tendency between compatibles and incompatibles shown in flower production by the manures is responsible. This view is supported by the rather higher value of $\frac{V_1}{V_2}$ in the compatibility/manure interaction for actual set of cherelles than in the proportionate setting table. The calculation of setting on a flowering basis of course eliminates flowering differences and equally eliminates flowering trends in relation to manures.

Percentage Wilt of Cherelles.

| Plot. | Control sub-plot | | P sub-plot | | K sub-plot | | PK sub-plot | |
|---|---|---|---|---|---|---|---|---|
| | Compatible. | Incompatible. | Compatible. | Incompatible. | Compatible. | Incompatible. | Compatible. | Incompatible. |
| 1A ... ... ... | 64.8 | 46.0 | 66.3 | 38.4 | 45.9 | 43.7 | 69.9 | 58.7 |
| 2D ... ... ... | 70.7 | 47.0 | 65.5 | 79.2 | 68.4 | 42.7 | 75.5 | 23.5 |
| 3E ... ... ... | 67.7 | 17.6 | 56.4 | 55.1 | 66.5 | 55.7 | 51.9 | 43.7 |
| 4B ... ... ... | 61.1 | 50.0 | 74.9 | 52.4 | 69.7 | 25.0 | 64.5 | 62.2 |
| 5F ... ... ... | 75.3 | 78.6 | 71.3 | 50.0 | 68.2 | 47.1 | 65.6 | 50.0 |
| 6C ... ... ... | 64.5 | 47.0 | 74.0 | 42.4 | 44.6 | 35.8 | 66.4 | 25.0 |
| Totals ... ... | 404.1 | 286.2 | 408.4 | 317.5 | 363.3 | 250.0 | 393.8 | 263.1 |

| Variance due to | Degrees of Freedom. | Sum of Squares. | Mean Square. | $V_1/V_2$ | $\frac{1}{2}\log_e V_1/V_2$ | P |
|---|---|---|---|---|---|---|
| Total ... ... ... | 47 | 11,100.6 | | | | |
| Whole sub-plots ... ... | 23 | 3,624.4 | 157.6 | | | |
| Plots ... ... ... | 5 | 970.9 | 194.2 | | | |
| K ... ... ... | 1 | 440.0 | 440.0 | 3.224 | 0.5853 | > 0.05 |
| P .... ... ... | 1 | 130.6 | 130.6 | | | |
| PK ... ... ... | 1 | 13.8 | 13.8 | | | |
| Error (*a*) ... ... | 15 | 2,065.1 | 137.7 | | | |
| Compatibles *v.* incompatibles | 1 | 4,271.4 | 4,271.4 | 27.138 | 1.6504 | < 0.01 |
| Compatibility/manures ... | 3 | 56.5 | 18.8 | | | |
| Error (*b*) ... ... | 20 | 3,148.3 | 157.4 | | | |

The difference between self-compatibles and self-incompatibles with regard to wilting of cherelles is highly statistically significant.

The effect of plots is insignificant, indicating that there is a tendency towards uniform percentage wilting of cherelles on all plots. Reference to the figures for Plot 5F will show that cherelle wilt was most severe on this plot—possibly connected with the lack of adequate shade upon the plot.

Potash effect is noticeably high, but rather below the lower limit of significance. The totals for manurial treatments show that potassium is beneficial in promoting less wilt, phosphorus having little or no effect in this direction. The very insignificant value ascribable to compatibility and manure interaction indicates that the two elements exercise no discriminatingly beneficial effect upon either class of tree. Both compatibles and incompatibles tend towards reduced wilting with potash application in the same degree.

Pods Harvested Per Tree.

| Plot. | Control sub-plot | | P sub-plot | | K sub-plot | | PK sub-plot | |
|---|---|---|---|---|---|---|---|---|
| | Com-patible. | Incom-patible. | Com-patible. | Incom-patible. | Com-patible. | Incom-patible. | Com-patible. | Incom-patible. |
| 1A ... ... ... | 7.86 | 7.60 | 11.00 | 2.67 | 16.67 | 14.00 | 10.78 | 20.00 |
| 2D ... ... ... | 11.67 | 0.00 | 3.60 | 1.50 | 7.50 | 6.00 | 7.14 | 6.50 |
| 3E ... ... ... | 12.80 | 3.00 | 9.50 | 7.83 | 8.80 | 3.50 | 10.28 | 12.33 |
| 4B ... ... ... | 5.00 | 0.83 | 5.00 | 4.67 | 3.33 | 2.67 | 8.50 | 7.25 |
| 5F ... ... ... | 1.44 | 0.50 | 2.33 | 0.00 | 8.83 | 2.33 | 3.17 | 0.50 |
| 6C ... ... ... | 8.57 | 0.00 | 7.43 | 1.80 | 5.33 | 1.50 | 2.00 | 0.50 |
| Totals ... ... | 47.34 | 11.93 | 38.86 | 18.87 | 50.46 | 30.00 | 41.87 | 47.08 |

| Variance due to | Degrees of Freedom | Sum of Squares. | Mean Square. | $V_1/V_2$ | $\frac{1}{2}\log_e V_1/V_2$ | P |
|---|---|---|---|---|---|---|
| Total ... ... ... | 47 | 1,026.0 | | | | |
| Whole sub-plots ... ... | 23 | 709.4 | 30.8 | 2.360 | 0.4278 | 0.05 |
| Plots ... ... ... | 5 | 452.1 | 90.4 | 6.919 | 0.9671 | < 0.01 |
| K ... ... ... | 1 | 58.1 | 58.1 | 4.445 | 0.7459 | 0.05 |
| P ... ... ... | 1 | 0.9 | 0.9 | | | |
| PK ... ... ... | 1 | 2.3 | 2.3 | | | |
| Error (*a*) ... ... | 15 | 196.0 | 13.1 | | | |
| Compatibles *v.* incompatibles | 1 | 105.2 | 105.2 | 15.030 | 1.3535 | < 0.01 |
| Compatibility/manures ... | 3 | 71.1 | 23.7 | 3.376 | 0.6084 | > 0.01 < 0.05 |
| Error (*b*) ... ... | 20 | 140.3 | 7.0 | | | |

The effect of plots is very significant. Of all the six plots, 1A is easily the best, yielding approximately eleven pods per tree; plot 5F is poor, giving less than two and a half pods per tree.

Manurial effects are significant. Potassium has a statistically significant effect upon the numbers of pods harvested and may be the final expression of the beneficial effects, induced by potassium, of increased flowering and reduced wilting.

The difference in harvested pod yields for compatible and for incompatible trees is statistically significant to a marked degree. The interaction between manures and compatibility is also significant, as can be seen from the steadily rising totals for pods harvested from the self-incompatibles towards the PK end, whilst the totals for the self-compatible members remain more or less stationary.

One effect of eliminating differential flower production by expressing pods on a percentage basis, is to remove much of the significant effect of potash noted in the previous table. It must be concluded, therefore, that the enhanced pod production resulting from application of potash is due fundamentally to enhanced flower production, together probably with reduced cherelle wilt.

The self-compatibles again differ significantly from the self-incompatibles, the ratio of the variances being much higher than the same ratio in the previous table. This is to be expected, since in the above table the superiority of the self-incompatibles over the self-compatibles in the matter of flower production is eliminated.

Pods Harvested Per 10,000 Flowers Borne.

| Plot. | Control sub-plot | | P sub-plot | | K sub-plot | | PK sub-plot | |
|---|---|---|---|---|---|---|---|---|
| | Compatible. | Incompatible. | Compatible. | Incompatible. | Compatible. | Incompatible. | Compatible. | Incompatible. |
| 1A ... ... ... | 13.9 | 13.5 | 12.9 | 3.7 | 77.9 | 10.1 | 17.2 | 9.8 |
| 2D ... ... ... | 60.6 | 0.0 | 8.6 | 5.1 | 35.2 | 6.4 | 41.4 | 14.7 |
| 3E ... ... ... | 28.2 | 6.5 | 22.5 | 11.0 | 13.3 | 10.1 | 36.2 | 13.5 |
| 4B ... ... ... | 7.8 | 4.0 | 23.0 | 20.2 | 50.9 | 2.5 | 40.9 | 7.0 |
| 5F ... ... ... | 15.1 | 0.3 | 6.4 | 0.0 | 17.0 | 4.7 | 13.3 | 0.9 |
| 6C ... ... ... | 29.1 | 0.0 | 24.6 | 3.8 | 15.0 | 4.6 | 8.8 | 9.7 |
| Totals ... ... | 154.7 | 24.3 | 98.0 | 43.8 | 209.3 | 38.4 | 157.8 | 55.6 |

| Variance due to | Degrees of Freedom. | Sum of Squares. | Mean Square. | $\frac{V_1}{V_2}$ | $\frac{1}{2}\log_e\frac{V_1}{V_2}$ | P |
|---|---|---|---|---|---|---|
| Total ... ... ... | 47 | 12,574.5 | | | | |
| Whole sub-plots ... ... | 23 | 4,457.2 | 193.8 | | | |
| Plots ... ... ... | 5 | 1,391.8 | 278.4 | 1.639 | 0.2470 | > 0.05 |
| K ... ... ... | 1 | 410.1 | 410.1 | 2.414 | 0.4406 | > 0.05 |
| P ... ... ... | 1 | 106.5 | 106.5 | | | |
| PK ... ... ... | 1 | 0.2 | 0.2 | | | |
| Error (*a*) ... ... | 15 | 2,548.6 | 169.9 | | | |
| Compatibles *v.* incompatibles | 1 | 4,364.4 | 4,364.4 | 35.282 | 1.7817 | < 0.01 |
| Compatibility/manures ... | 3 | 1,279.9 | 426.6 | | | > 0.01 |
| | | | | 3.449 | 0.6190 | < 0.05 |
| Error (*b*) ... ... | 20 | 2,473.0 | 123.7 | | | |

## Conclusions.

(1) Throughout the whole of the analyses, the differences between self-compatibles and self-incompatibles are statistically significant to a high degree. In setting of cherelles and in production of pods, the self-compatible tree is superior to its self-incompatible neighbour: in the production of flowers and in retention of cherelles the self-compatible is inferior. The initial advantage which the self-compatibles gain in their having a greater proportion of flowers setting to cherelles is maintained throughout the whole cycle from setting to harvesting, in spite of the greater losses in cherelles, from wilt, sustained by the self-compatibles.

(2) Manurial effects are indefinite. Potash does seem, however, to be more beneficial than phosphorus in all stages of pod production; yet only in consideration of harvested pods is the effect of potash statistically significant. The first stage at which potash approaches towards a

significant effect is in reducing the amount of cherelle wilt, resulting therefore in a correspondingly increased number of cherelles which win through to become mature pods. On these six plots under observation, therefore, lack of adequate potash supply is one of the factors contributing to heavy cherelle loss by wilting.

(3) The significance ascribable to interaction between manures and compatibility seems to rise towards the end stages in pod production. Self-incompatibles receive more benefit from the application of manures than do the self-compatibles. The reason for this discrimination between the two classes of tree is difficult to explain, since its origin cannot significantly be found either in flowering or in setting and most certainly not in wilting.

## Discussion.

The recognition of the incidence of self-incompatibility amongst a considerable proportion of the Trinidad cacao population led to the consideration that it might exert a depressing effect upon the yields of cacao obtained from fields containing such an admixture of self-compatible trees and self-incompatible trees. The results of this and the preliminary paper (3) have shown that this expectation is to a large extent met in practice.

At the outset, it will be in order to indicate the proportion of the two classes of tree as they occur on the six plots under observation. The figures do not necessarily correspond with those calculated from Table II, since guard row trees are included :

| Plot. | Per cent. self-compatible. | Per cent. self-incompatible. |
|---|---|---|
| 1A ... | 47 | 53 |
| 2D ... | 76 | 24 |
| 3E ... | 51 | 49 |
| 4B ... | 46 | 54 |
| 5F ... | 61 | 39 |
| 6C ... | 44 | 56 |

The seedling progenies of plots 1A, 3E, 4B and 6C are from open pollinated pods from four of the self-incompatible Imperial College Selections at River Estate, I.C.S. 100, I.C.S. 4, I.C.S. 5 and I.C.S. 2 respectively. It is known with a high degree of certainty that a self-incompatible tree will set fruit only with pollen from a tree which is itself self-compatible. Hence the trees on these four plots are the progenies of self-incompatible, self-compatible crossings. It is of interest to note that on these four plots, too, the proportions of self-compatible and self-incompatible seedlings are approximately equal.

Voelcker (2) has shown conclusively that on self-compatible trees the pods which set may be either from selfings, crossings or a combination of both. The chances, therefore, of a self-compatible, self-compatible mating taking place on a self-compatible tree are higher than the chances of a self-compatible, self-incompatible cross. Assuming compatibility and incompatibility to be determined genetically, there would be an expectation for preponderance of self-compatible offspring amongst the progeny from an open pollinated pod from a self-compatible tree. Reference to the figures for plots 2D and 5F, seedling progenies from the self-compatible I.C.S. 6 and I.C.S. 1 respectively, does indeed show this to be the case.

The figures given in the first part of the paper show that the self-compatible tree yields a final average harvest of 7.53 pods, whilst the self-incompatible tree produces only 4.37 pods. Taking into consideration the pods harvested from the two classes of tree, together with the relative proportions of both types, for each of the six plots, the increase in yields which would be obtained by replacing the self-incompatibles with self-compatibles from the same plot can readily be calculated. For the respective plots they are :—1A 3.6 per cent. : 2D 12.1 per cent. : 3E 24.7 per cent. : 4B 27.2 per cent. : 5F 36.5 per cent. : 6C 89.4 per cent. The interesting fact is that the calculated increase in possible yield rises progressively from plot 1A to plot 6C and can be correlated, perhaps, with the decreasing soil fertility known to exist. In other words, on a poor soil the contrast between self-compatible and self-incompatible trees is more striking than on a good soil. How far this hypothesis could be generally applied is doubtful. Support for it is shown in the significant interaction between compatibility and manures when final pod yields are taken into account ; the self-incompatible tree is more beneficially affected by manures than is the self-compatible tree.

A calculation to include all the trees of the six plots shows that the average increase in yield which could be expected, were the self-incompatible trees replaced by self-compatible trees, is only 24 per cent. This figure applies, of course, only to the two acres of young seedling trees which have been under observation. With other populations, on different soil-types and under different climatic conditions, and, perhaps particularly, of different ages, the figures might be very different.

Indeed, at the present stage in these investigations, any statement about the interaction of compatibility and final yield must be cautiously made and cautiously received. There is equal danger in regarding incompatibility as a negligible cause of low yields and in hailing it as the major cause. Imperial College Selections occurring in both classes prove that under certain conditions an individual self-incompatible tree may be as good as any self-compatible. In populations there are many factors besides the intrinsic yielding powers of individuals which may modify performance ; these possibly include closeness of planting or the density of canopy due to age or to other factors, as well as the proportions and distribution of self-compatibles acting as efficient

pollinators for the incompatibles. Further information will become available in time, both from the trees under observation in this experiment and from more recent clonal plantings. Meanwhile, there is no doubt, from the practical standpoint, that the best planting material is that which is self-compatible throughout, since all hazards attendant upon the necessity for cross-pollination would then be eliminated.

Using the knowledge that self-compatibles and self-incompatibles occur with approximately similar frequencies on the plots, it would be justifiable to assume that there will be available approximately equal amounts of pollen from the two classes of tree. The self-compatible tree will set with pollen either from a self-compatible tree or from a self-incompatible tree; the self-incompatible tree, however, is dependent upon the deposition of pollen from a self-compatible tree before setting can ensue. Hence the self-compatible tree would set twice as many flowers from randomised cross-pollination as the self-incompatible tree. It has been calculated, however, in an earlier part of this paper, that the self-compatible tree sets three and a half times as many flowers as the self-incompatible tree. This excess over the doubled rate of flower setting which, from a consideration of cross-pollination alone, would obtain amongst the self-compatibles can be attributed to the success of self-pollinations on the self-compatibles and lack of success of self-pollinations on the self-incompatibles. Of the seven hypothetical flowers on a self-compatible tree which set, four will be set from cross-pollination and three from self-pollination, while the two settings on the self-incompatible tree can be only from cross-pollination. Hence there will be rather more pods, on the self-compatible tree, produced by cross-pollinations than produced by self-pollinations. This result stands in corroboration of Voelcker's (2) finding that on self-compatible trees up to 50 per cent. of the pods arise from cross-pollinations. The desirability of establishing the nature of the agent of pollination in cacao needs no further emphasis.

The consistently greater degree of cherelle wilt amongst the self-compatibles than amongst the self-incompatibles is suggestive of a physiological reaction in the tree against an overproduction of young cherelles. There appears to be a close connection between heavy setting of young cherelles and heavy wilting. It would seem, therefore, that the competition for nutrients and water, which must undoubtedly exist amongst numerous cherelles, is the prime cause of cherelle wilt, the intensity of which will be influenced by the general physiological condition of the tree. If this general physiological condition can be in any way improved, the number of cherelles which win through to become mature pods will be increased. Addition of potash to a soil naturally deficient in this element tends to increase the number of harvestable pods, chiefly by reducing the number of cherelles which wilt. It is very noticeable from Table III (*a*) that for both classes of tree, plot 5F is the worst as regards intensity of cherelle wilt; this plot suffered from, and still suffers from, a lack of shade, a condition which undoubtedly tends to lower the physiological well-being of the trees.

It would seem, therefore, that there is a definite limit to the number of cherelles which the tree has power to mature. Any super-abundance of cherelles—and this would naturally occur most frequently on the self-compatible tree—will automatically lead to a higher proportionate loss of cherelles. Any factor enhancing the tree's physiological status will decrease the percentage wilt.

## Summary.

(1) Records of observation on six plots of young seedling cacao, all open pollinated progenies, with regard to flowering intensity, setting of young fruits, wilting of fruits and final pod yields, are given with reference to the incidence of self-compatibility and self-incompatibility.

(2) The results are treated statistically.

(3) The self-compatible tree is shown to be superior in final yield of harvested pods to the self-incompatible tree. The self-incompatible tree produces more flowers, sets far fewer cherelles, wilts a smaller proportion of cherelles and yields a smaller return of mature pods, in comparison with the self-compatible tree.

(4) Attempts to analyse the effects of manures upon the various stages which make yield possible give no statistically significant results, except that potash is beneficial in enhancing pod yields. The self-incompatible element of the population is benefited more than the self-compatible element by the application of manures.

In conclusion the author wishes to acknowledge his indebtedness to Mr. O. J. Voelcker for collaboration in the early stages of the work, to Professor E. E. Cheesman for his encouragement and interest, to the Department of Agriculture, Trinidad, for facilities and to Mr. D. D. Paterson, Reader in Agriculture at the Imperial College of Tropical Agriculture, for help in statistical matters.

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# A NOTE ON THE RANGE OF COMPATIBILITY IN CACAO.

By F. W. Cope.

During the season 1937-38 hand self-pollinations were made on the trees of six blocks of seedling cacao at River Estate (4) (1). Each plot was tested about once a month, the work being accelerated by the omission of the obviously self-incompatible members, and tests restricted to those trees showing self-compatibility.

Only in the early tests were more than ten flowers per tree self-pollinated at each visit. The number of tested flowers per tree was later reduced to ten per visit, or failing the requisite number, as many fresh flowers as possible. The young cherelles resulting from self-compatible hand self-pollinations were counted and recorded fourteen days after the pollinations.

It was therefore possible to follow the course of the reactions to self-pollinations of individual trees and, collectively, of whole plots. A considerable variation, both between trees on the same date, and between subsequent tests on the same tree, soon became apparent.

A range of compatibility amongst the young trees can readily be established from the individual tree records so obtained. Some trees quickly became recognizable as those on which hand self-pollinations would give a consistently high proportion of young cherelles set. Others, again, soon became readily distinguishable as trees which would set only a low proportion of the flowers so tested. There manifested itself, also, a group of trees which consistently set nothing from hand self-pollinations and therefore truly self-incompatible.

On any tree, furthermore, the success of self-pollinations varied, within limits, throughout the year, all the trees tested exhibiting the same trend.

The figures quoted in Tables IV and V will make these variations clear.

From Table IV and from Graph I constructed from the table it will readily be seen that the major bulk of the self-compatible trees comprises those with reactions to hand self-pollinations giving 50 to 20 per cent. successes. Four sets at fourteen days from ten pollinations would seem to be the modal chance of success; this is, of course, the integrated effect over the whole year.

The group of 152 trees under the heading O were those trees which consistently refused to set a single cherelle from at least four different tests and from at least ten flowers.

The 59 trees falling in the ten to zero percentage class set, on the average, one cherelle from 29 hand selfings. This ratio is very much greater than the corresponding flower to cherelle ratio for natural settings on self-incompatible trees; reference to Table III (*a*) shows that on the self-incompatible tree only one cherelle sets as a result of natural pollination from about 400 flowers. It must be concluded, therefore, that these 59 trees do show extremely feeble compatibility. Voelcker (2) (3) found that on rare occasions setting could be induced on self-incompatible Imperial College Selections. In the first paper of this report, these 59 trees have been classed amongst the self-incompatibles: any tree setting two or more cherelles from ten pollinations, providing it behaved similarily at subsequent tests, is classed as self-compatible. It is, however, easy to distinguish from the records those trees which are genuinely self-compatible from those which are self-incompatible.

Voelcker (3), working on the mature Imperiai College Selections at River Estate, came to the conclusion that there is a seasonal variation in the success attendant upon self-pollinations on self-compatible trees. This observation has been confirmed by the extensive tests forming the subject of this paper.

**Table IV.**

Numbers of Trees which fall within the several Rangers of Compatibility, as measured by the success of hand self-pollinations.

| Percentage of selfed flowers which set | 100-70 | 70-60 | 60-50 | 50-40 | 40-30 | 30-20 | 20-10 | 10- 0 | 0 |
|---|---|---|---|---|---|---|---|---|---|
| Number of trees ... | 4 | 14 | 33 | 56 | 75 | 64 | 28 | 59 | 152 |

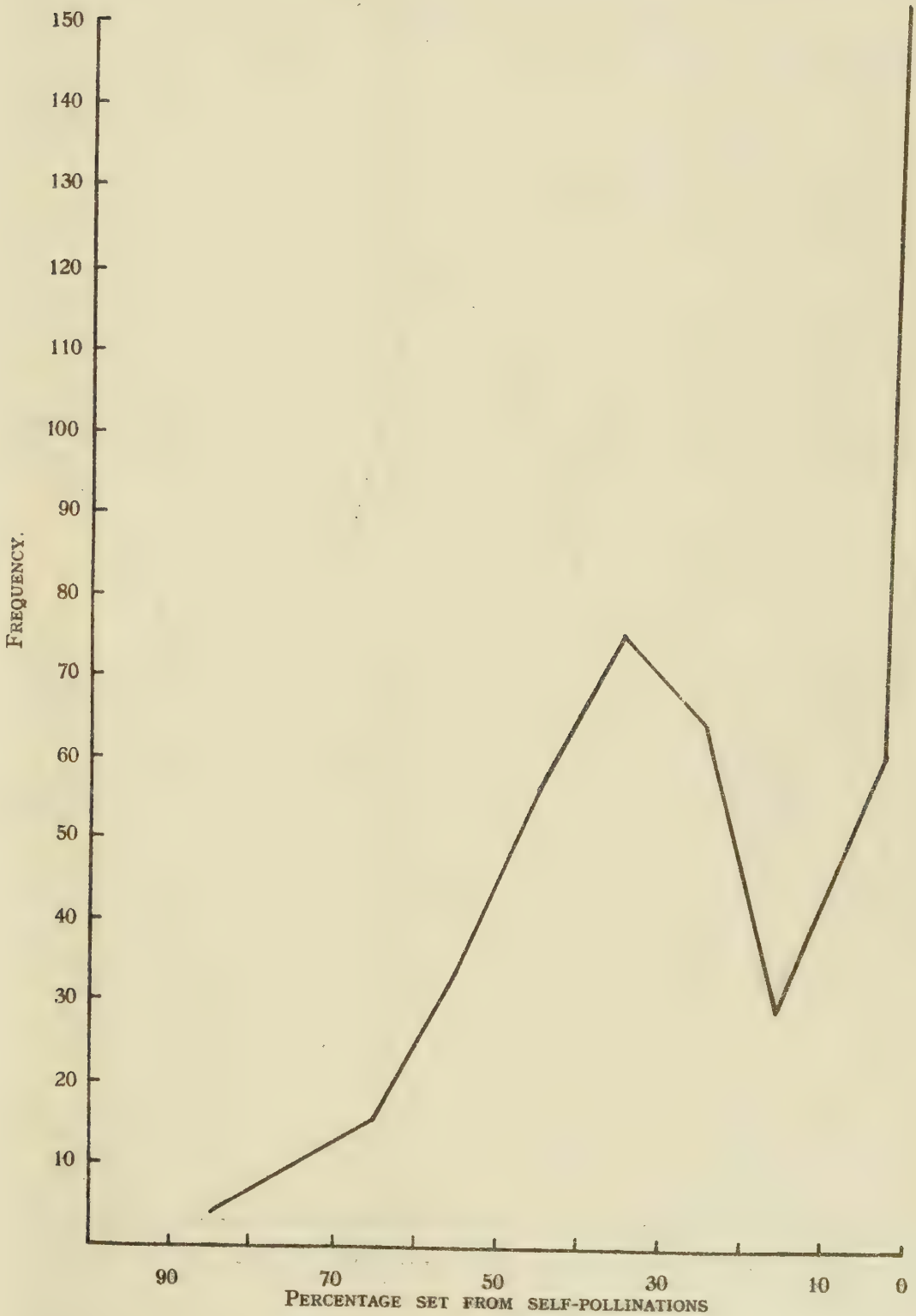
**Table V.**

Seasonal Variations in Percentage Settings from Hand Self-Pollinations.

| Month. | Aug. '37. | Sept. | Oct. | Nov. | Dec. | Jan. '38. | Feb. | Mar. | Apr. | May. | June. | July. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| % set ... ... | 17.6 | 33.3 | 22.8 | 36.7 | 36.2 | 55.8 | 57.3 | 38.5 | 40.0 | 35.0 | 43.1 | 47.2 |

GRAPH I

FREQUENCY GRAPH OF PERCENTAGE SUCCESSES FROM HAND SELF-POLLINATIONS.



# GRAPH II

MONTHLY VARIATIONS IN SUCCESSES FROM

HAND SELF-POLLINATIONS.

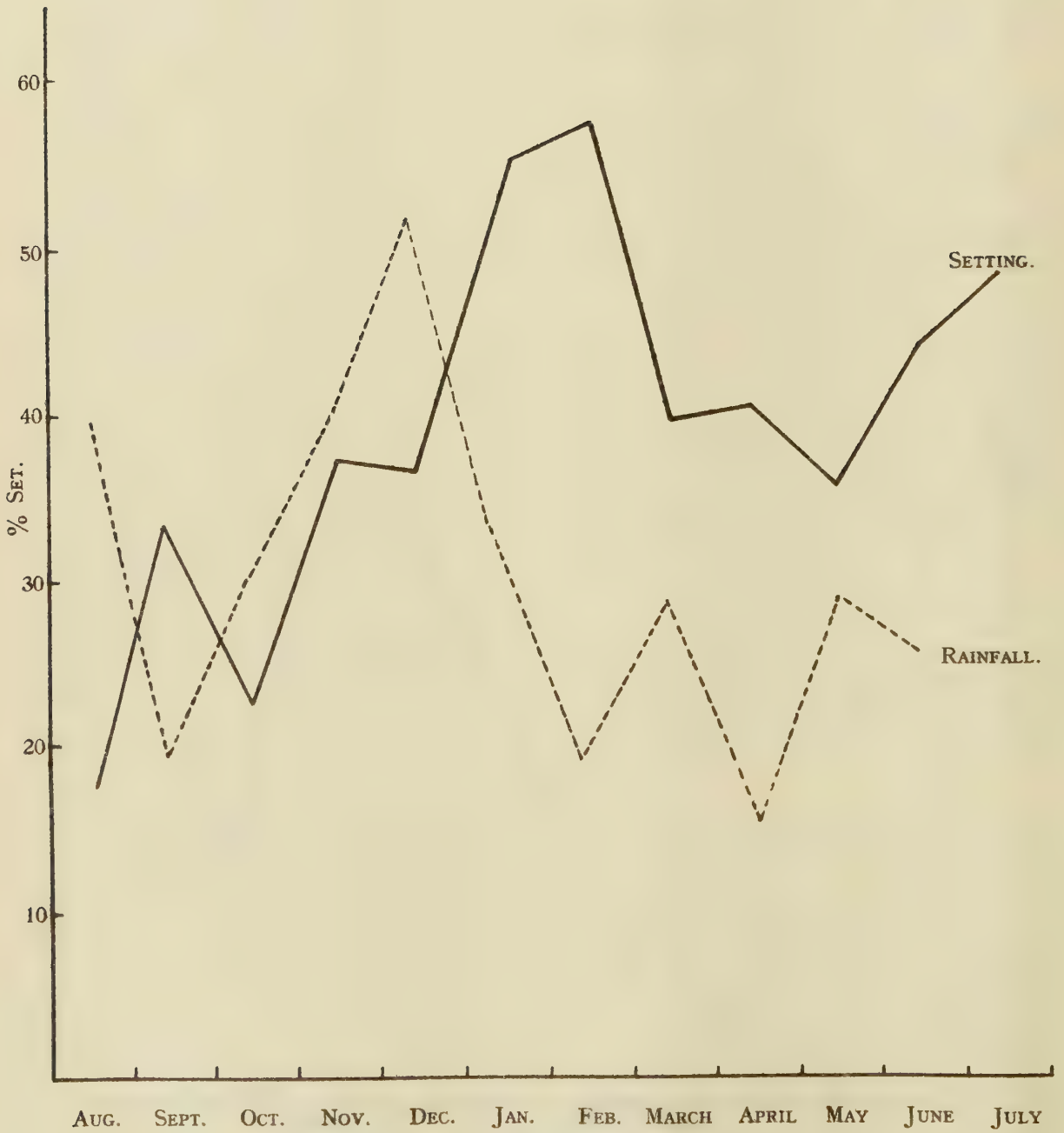


Table V gives the monthly percentages of flowers setting to young cherelles at 14 days after self-pollinations. Only the self-compatible members of the six plots are considered.

The Table V and Graph II constructed from the table demonstrate a variation from month to month in the percentage successes resulting from hand self-pollinations on self-compatible trees. Lack of success during the first month of August may have been due to a still imperfect technique ; but thereafter no variation in result can be attributed to careless pollination. The most successful results were obtained in January and February.

There seems to be no complete correlation between setting and monthly rainfall, the curve for which is added in the graph. But periods of low rainfall correspond roughly with periods of high percentage set. It is suggested that the climatic conditions at the time of pollination are of considerable influence in determining the numbers of cherelles which result from hand selfings. Rainfall immediately preceding or following hand-pollinations invariably led to low percentage setting, while the best results were obtained during short spells of fine days.

**Summary.**

(1) The success of hand self-pollinations varies from tree to tree, some self-compatibles setting very easily and others less readily from hand self-pollinations. There appears to be, therefore, a range of self-compatibility.

(2) The success of hand self-pollinations varies from time to time during the year.

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## COMPATIBILITY AND FRUIT SETTING IN CACAO.

By F. W. Cope.

The inability of a large proportion of the cacao trees of Trinidad to set fruit with their own pollen has now been recognized for seven years (4) (5). It was more recently proved that such trees adhere rigidly to their self-incompatible condition the whole year round (7), contradicting the earlier supposition that self-incompatibility was seasonal in its occurrence (6).

Satisfactory setting on self-incompatible trees has only been achieved by the deposition of pollen from a self-compatible tree upon the stigmas of flowers of those trees (7). Sporadic settings from cross-pollinations betwen self-incompatible trees have been noted (6) (3) (7), and it was decided to compare the behaviour of self-incompatible trees towards self- and cross-pollinations, both with pollen from self-incompatible and from self-compatible trees.

Pollinations of this nature were therefore undertaken on the trees of six seedling plots (8) (1) at River Estate. Only those self-incompatible trees were selected for testing which had, from previous records (8) (1) shown themselves to be either completely self-incompatible or else extremely feebly self-compatible, as manifested by their very occasional setting from hand self-pollinations (2). There were, for instance, eleven trees tested on Plot 1A, and the pollinations were always done in the same way ; tree 1A 5, at any one visit, would have five flowers self-pollinated, five flowers cross-pollinated from flowers of the next tested tree, 1A 8, and finally five flowers cross-pollinated from the nearest self-compatible tree, 1A 7. When these 15 pollinations had been completed, tree 1A 8 would then receive similar treatments, self-incompatible pollen from 1A 12 and self-compatible pollen from 1A 9 being used in the respective cross-pollinations.

Each of the flowers belonging to one set of pollinations was tagged with the same colour wool. Three days later the number of flowers still on were counted and after a lapse of 14 days from pollination the number of cherelles arising from each set of pollinations were separately recorded.

The experiments were carried out for a space of six months (8.7.38 to 9.1.39).

**Experimental Results.**

The bulky mass of results is presented in a concise form in Table VI, which shows the proportion of settings arising from each of the different types of pollination for each of the six plots. The numerator gives the actual number of cherelles set at 14 days, the denominator the the number of flowers pollinated. In addition the bottom row of figures gives the percentage of the pollinated flowers which remained on at three days from pollination

**Table VI.**

| Type of pollination. / Plot. | Self-incompatible selfings. | Self-incompatible self-incompatible crosses. | Self-incompatible self-compatible crosses. |
|---|---|---|---|
| 1A | 5/386 | 3/382 | 116/372 |
| 2D | 0/139 | 3/131 | 39/135 |
| 3E | 3/476 | 8/469 | 172/463 |
| 4B | 3/192 | 15/184 | 52/184 |
| 5F | 1/102 | 3/96 | 25/101 |
| 6C | 3/182 | 3/181 | 54/167 |
| Totals | 15/1477 | 35/1443 | 458/1422 |
| Percentage success | 1.02 | 2.43 | 32.2 |
| Percentage flowers on at 3 days | 25.9 | 27.3 | 53.1 |

From the results it can be seen that cross-pollinations between self-incompatible trees result in a setting of cherelles nearly two and a half times as great as self-pollinations on the same trees; yet this increased degree of setting is still extremely small and in a mixed population of compatibles and incompatibles the amount of setting resulting from cross-pollinations between self-incompatible trees would be almost negligible.

At three days from pollination about 75 per cent. of the flowers receiving incompatible pollinations fall, while the flowers receiving self-compatible pollen drop only to the extent of some 50 per cent., and thereafter the loss of flowers is relatively small.

These figures bear out Pound's conclusion (4) (5) and those of Voelcker (7) that self-incompatible trees crossed by self-incompatible trees set only very poorly, but when crossed by self-compatible trees the set is perfectly satisfactory.

### Correlations between Flowering Intensity and Setting of Cherelles.

From a consideration of the above results, it is very obvious that the vast majority of the pods on any self-incompatible tree must be the result of crossings from self-compatible trees. How far the degrees of association between self-compatibles and self-incompatibles would affect the yields of the latter class of tree in a mixed population is unknown. A knowledge of the habits of the insect or insects responsible for cross-fertilizations in cacao would be of great assistance in elucidating this point.

Some attempt has been made, however, to estimate the dependence of self-incompatible trees upon the proximity of self-compatible trees. On the six progeny seedling plots at River Estate there exists a population of young trees, carefully spaced in planting, of which the intensities of flowering and of setting of cherelles are known (8) (1), together with the identification of individual trees as self-compatible or as self-incompatible.

The yield of any self-incompatible tree will ultimately depend upon the numbers of its flowers which receive self-compatible pollen; this, in turn, will depend upon two factors, the first being the actual number of flowers produced on the self-incompatible tree and the second being the intensity of cross-pollination. A self-incompatible tree which produces only very few flowers will be at an obvious disadvantage compared with a heavily flowering tree, provided the frequency of insect visits is the same for both trees. In the same way, a self-incompatible tree which receives more insect visitors bearing self-compatible pollen will set more abundantly than a similar tree receiving only very few pollinators. There will be, therefore, a dependence of crop production upon the numbers of flowers the self-incompatible tree produces and the density of distribution of self-compatible pollen.

The number of pods borne or number of cherelles set will be proportional to the product of flowering intensity and density of pollen distribution. This consideration arises from the mathematical law that if a quantity A is dependent upon two quantities B and C, it is proportional to the product of those two quantities.

It was considered that the density of self-compatible pollen for any self-incompatible tree would probably be proportional to the number of self-compatible flowers borne on trees immediately adjoining the tree in all directions. A plan of each of the six plots was made, therefore, and self-compatible and self-incompatible trees marked on it. The self-incompatible members (ignoring those occurring on the margins) were listed and their yearly flowering intensities appended. From the plan it was possible to determine, for each individual self-incompatible tree, how many of the eight immediately adjacent trees were self-compatible and having determined this, to estimate the number of flowers borne on these self-compatible trees. The table was completed by adding the number of cherelles set on the self-incompatible tree in question; the table for plot 5F is given in Table VII.

**Table VII.**

CHERELLES SET, FLOWERS BORNE, AND NUMBERS OF SURROUNDING SELF-COMPATIBLE FLOWERS FOR THE SELF-INCOMPATIBLE TREES OF PLOT 5F.

| Self-incompatible tree number. | Cherelles set. | Flowers borne. | Surrounding self-compatible flowers. | Product of floral numbers. |
|---|---|---|---|---|
| 5F 18 | 5 | 3490 | 3610 | $125989 \times 10^2$ |
| 23 | 21 | 18340 | 8330 | 1527722 |
| 24 | 9 | 4020 | 14030 | 564006 |
| 25 | 4 | 10850 | 12100 | 1312850 |
| 26 | 9 | 5160 | 16320 | 842112 |
| 27 | 12 | 7160 | 11120 | 796192 |
| 30 | 0 | 640 | 9600 | 61440 |
| 33 | 1 | 620 | 12270 | 76076 |
| 34 | 0 | 680 | 8490 | 57732 |
| 39 | 8 | 2980 | 19360 | 576928 |
| 42 | 0 | 1060 | 9320 | 98792 |
| 46 | 10 | 3070 | 10320 | 316824 |
| 49 | 0 | 810 | 25770 | 208737 |
| 55 | 1 | 1220 | 6860 | 83692 |
| 65 | 28 | 16820 | 10230 | 1720686 |
| 71 | 0 | 6530 | 8180 | 534154 |
| 72 | 4 | 2900 | 2110 | 61190 |
| 73 | 6 | 5760 | 1810 | 104256 |
| 88 | 4 | 1870 | 17150 | 320705 |

Correlations were then calculated between "cherelles set" and "flowers borne" and between "cherelles set" and "product of floral numbers". The correlation coefficients for the six plots are given below in Table VIII.

**Table VIII.**

CORRELATION COEFFICIENTS FOR PLOTS 1A TO 6C. Self-incompatibles.

| Plot. | Cherelles/flowers borne. | Cherelles/product of floral numbers. |
|---|---|---|
| 1A ... | +0.7370 | +0.4698 |
| 2D ... | +0.8049 | +0.6419 |
| 3E ... | +0.5063 | +0.4347 |
| 4B ... | +0.7087 | +0.5973 |
| 5F ... | +0.8372 | +0.8158 |
| 6C ... | +0.3530 | +0.2062 |
| 1A-6C ... | +0.6612 | +0.5144 |

Table VIII indicates a consistently higher degree of correlation between the number of cherelles a self-incompatible tree sets and the number of flowers it bears than between the number of cherelles set and the product of the number of its own flowers and of surrounding compatible flowers. It may therefore be concluded that pollinations on a self-incompatible tree are not confined to the pollen from immediately adjacent self-compatible trees but that the distribution of self-compatible pollen tends to be uniform. Presumably, therefore, the pollen vector is not restricted to tree to adjacent tree migrations, but once having visited any particular tree may fly considerable distances before visiting a second. Pods borne on a self-incompatible tree may therefore have arisen from pollinations from a self-compatible tree more remote than its immediate neighbours. Evidence to support the view that the pollen vector may visit flowers widely separated in space has been obtained from a cursory study of some Criollo buddings on San Juan Estate, Trinidad. On these trees, which bear usually pods with only white beans, there occur occasionally pods in which one to many, or sometimes all, of the beans are purple, indicating pollination, partially or completely, with pollen from a Forastero tree. The Forastero stocks may be ruled out as a possible source of pollen since the cultivation is so cleanly maintained; the nearest trees of this type are situated at least fifty yards away. Further studies on these trees are contemplated and should yield valuable results.

It is of considerable interest to note that the two highest degrees of correlation occur on those plots where there is a predominance of self-compatible trees, plots 2D and 5F (1). Owing to the numerous centres of self-compatible pollen supply, the density of pollen distribution on these two plots will tend to be more uniform than on those other four plots where the self-compatible trees are less frequent. On plots 2D and 5F every individual self-incompatible tree is subjected to a more or less uniform pollen distribution and will tend, therefore, to set cherelles in proportion to the numbers of flowers which it bears.

After clearing the ground of the self-incompatible members of the six plots, the self-compatibles were then accorded the same analysis. Since a self-compatible tree will set equally well with pollen from either self-incompatible or self-compatible sources, the flowers borne on the eight immediately adjacent trees were summed and the totals entered into tables similar to that presented for the self-incompatibles in Table VIII. The coefficients of correlation for the six plots are given in Table IX.

**Table IX.**

CORRELATION COEFFICIENTS FOR PLOTS 1A TO 6C. Self-compatibles.

| Plot. | Cherelles/flowers borne. | Cherelles/product of floral numbers. |
|---|---|---|
| 1A ... | +0.8429 | +0.8764 |
| 2D ... | +0.3311 | +0.2520 |
| 3E ... | +0.8075 | +0.8199 |
| 4B ... | +0.6240 | +0.4807 |
| 5F ... | +0.7974 | +0.7177 |
| 6C ... | +0.7953 | +0.5409 |
| 1A-6C ... | +0.7249 | +0.6590 |

As amongst the self-incompatibles, the numbers of flowers on adjacent trees have little effect upon the intensity of cherelle setting on the self-compatible trees of the six plots. There is a high degree of correlation between setting and flowering intensity, showing that the distribution of pollen is more or less randomised, so that trees tend to set in proportion to the number of flowers they bear.

### Summary.

(1) Cross-pollinations made between self-incompatible trees are over twice as successful in result as self-pollinations.

(2) The setting of cherelles upon either self-compatible or self-incompatible trees is not so much dependent upon the potential supply of pollen from immediately neighbouring trees but rather upon each tree's individual flower production, indicating that the pollen vector may visit flowers widely separated in space.

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## STUDIES IN THE MECHANISM OF SELF-INCOMPATIBILITY IN CACAO—I.

By F. W. Cope.

A striking tree to tree variation in reaction to self-pollinations was discovered in cacao by Pound (3, 4). Some trees on which hand self-pollinations were performed lost all the flowers so treated within six days: others retained all the flowers self-pollinated, while some trees lost varying percentages of flowers.

Cross-pollinations on those trees which normally set only very poorly or nothing at all from self-pollinations were found materially to increase the percentage setting, provided the " foreign " pollen came from a tree itself capable of setting with its own pollen. Exactly similar phenomena are known to occur in other plants, where genetical inheritance has been shown to hold (5).

The cause of incompatibility in cacao is still unknown. Pollen tube failure, lack of gametic fusion, and the operation of lethals in the fertilised ovum might singly or severally contribute to the failure of self-pollinations in the self-incompatible tree.

This preliminary paper records the reactions to self- and cross-pollinations made on two trees, one self-compatible and the other self-incompatible. The work had as its object the proving of Posnette's (2) finding that the tubes of incompatible pollen may fail to grow down the style.

Nebel's method (1) for staining pollen tubes in the style was employed, with lacmoid and martius yellow as the staining agents. In all cases, the pollen was allowed to germinate on the style for five hours and the lengths of tubes then determined.

The self-pollinations and cross-pollinations were performed on a vigorous cutting of Imperial College Selection 101, known from several tests to be self-compatible, and on a seedling tree M1, known to be self-incompatible. Both trees grow close together on the " Saman " plot at the Imperial College. Six selfings and six crosses were made at each occasion on both trees.

After a lapse of five hours from pollination, the flowers were collected in four separate, labelled, tubes. Each flower of the six comprising one set of pollinations had its style severed as close to the ovary as possible; the style was mounted in a small drop of water on a clean slide and then squashed under a cover-slip. After removing as much water as possible, the slide or cover-slip, depending on whichever of these retained the squashed style, was flooded with Carnoy's fluid. After a thorough rinsing in water, the six styles from a set of pollinations were mounted together on a slide and stained with a solution containing 50 mg. of lacmoid and 50 mg. of martius yellow in 100 ccs. of water, rendered slightly alkaline with a few drops of ammonia solution.

Mounts were made in 50 per cent. glycerine containing a little lacmoid in solution.

The courses of the pollen tubes in the styles were rendered strikingly visible by this technique and it was a simple matter to select individual tubes and trace their courses on paper with the aid of a camera lucida. The use of a red filter, to accentuate the contrast between the blue-stained tube and yellow-stained style, was found advantageous.

Table X shows the average tube lengths (in arbitrary units) for each of the four sets of pollinations. A tube length of 100 units would be approximately to the base of the style. The full table giving measurements of at least three individual tubes in each of the twenty-four styles for every day would be too bulky, but has been used in the calculations of the analysis of variance found in this paper.

**Table X.**

AVERAGE DAILY POLLEN TUBE LENGTH FROM FOUR SETS OF POLLINATIONS.

Arbitrary Units. Five Hours After Pollination.

| Date. | M1 × ICS 101 | ICS 101 × M1 | M1 × M1 | ICS 101 × ICS 101 |
|---|---|---|---|---|
| 4.10.38 | 82 | 78 | 70 | 45 |
| 6.10.38 | 100 | 72 | 106 | 56 |
| 7.10.38 | 89 | 78 | 93 | 44 |
| 10.10.38 | 60 | 72 | 71 | 63 |
| 13.10.38 | 89 | 63 | 56 | 56 |
| 17.10.38 | 77 | 91 | 62 | 84 |
| 20.10.38 | 96 | 56 | 86 | 63 |
| 27.10.38 | 92 | 63 | 75 | 42 |
| 1.11.38 | 84 | 61 | 68 | 35 |
| 4.11.38 | 88 | 78 | 94 | 65 |
| 8.11.38 | 90 | 70 | 84 | 72 |
| 14.11.38 | 94 | 51 | 81 | 69 |
| 15.11.38 | 68 | 54 | 83 | 60 |
| Average ... | 86 | 65 | 81 | 69 |

The most striking result of this early experiment is to show that pollen on the styles of M1, a self-incompatible tree, grows faster than similar pollen on the styles of self-compatible I.C.S. 101. Whether it be pollen from a self-incompatible tree (selfings) or pollen from a self-compatible tree (crossings) seems to make little difference. Furthermore, the mating which is known to lead to a negative set, the selfing of M1, does not imply the slowest growth of the pollen tubes.

It may be concluded, therefore, that as far as these two experimental trees are concerned, the pollen effect is negligible: pollen tubes from M1 grow at only a very slightly lower rate than I.C.S. 101 pollen tubes—actual ratio, 155 to 146. The styles, however, exert markedly an effect upon the rates of pollen tube growths in them and the interesting result is that the M1 style permits the more rapid growth of the two—actual ratio, 167 to 134. These differences are tested for statistical significance by the analysis of variance: the stylar effect is shown to be highly significant.

Differences in tube growth on different days are also significant, but the order of significance is less than that for pollination treatments.

It would seem, then, from the preliminary experiments, that differential growth rates of pollen tubes in styles of self-compatible and self-incompatible flowers are not themselves responsible for the lack of setting in selfings on self-incompatibles. Stout and Chandler (6), working on Hemerocallis, came to similar conclusions regarding the pollen tube behaviour in self-incompatibles of that genus. Their researches showed that the inhibition towards pollen tube growth occurs actually in the ovary and not in the style. It might well be that in cacao the same antagonistic influence is exerted only in the ovary and not in the style. It is hoped that further work will elucidate this point. The work of Yasuda (7) corroborates the ovarian and placental inhibitions of pollen tube growth in incompatible matings.

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ANALYSIS OF VARIANCE.

| Variance due to | Degrees of Freedom. | Sum of Squares. | Mean Square. | $V_1/V_2$ | $\frac{1}{2}\log_e \frac{V_1}{V_2}$ | p |
|---|---|---|---|---|---|---|
| Total ... ... ... | 294 | 108,253 | | | | |
| Treatments ... ... | 3 | 33,773 | 11,285 | 53.355 | 1.9885 | < 0.01 |
| Days ... ... ... | 12 | 15,701 | 1,308 | 6.199 | 0.9122 | < 0.01 |
| Error ... ... ... | 279 | 58,779 | 211 | | | |
| Treatment: | | | | | | |
| Pollen effect ... ... | 1 | 30 | 30 | | | |
| Style effect ... ... | 1 | 32,633 | 32,633 | 154.66 | 2.5206 | < 0.01 |
| Interaction ... ... | 1 | 110 | 110 | | | |
| Error ... ... ... | 279 | 58,779 | 211 | | | |

## (B) CHEMICAL AND ECOLOGICAL SECTION.

## THE CHEMICAL PROGRAMME OF 1938.

By F. Hardy.

### Staff.

The work during the year 1938 was mainly in the hands of Dr. E. C. Humphries, who took up his appointment at the College in October, 1937. This officer had to devote most of his time during the past year to teaching, no second officer being appointed to the Chemistry Department until December, 1938. Thus, progress has again been slow because of reduced staff, and most of the experimental work has been biochemical, in continuation of the investigations begun by Mr. J. A. McDonald towards the end of 1935, and carried on by Dr. D. W. Duthie between October, 1936 and July, 1937.

### Nigerian Cacao Soils.

Soil investigations during the year have consisted mainly in a laboratory examination of 127 soil samples comprising 15 soil profiles representative of the cacao soils of Nigeria. These samples were kindly collected and sent to the College by Mr. H. C. Doyne, Chemist of the Nigerian Department of Agriculture. A brief article on the results of the analyses is included in this Annual Report. The Nigerian cacao soils closely resemble some of the Gold Coast soils described in the Annual Reports for 1936 and 1937, in that they are loose, sandy types, derived from non-calcareous, highly acidic materials formed by the weathering of Archaean gneissose rocks, though some have been formed from Tertiary sandy sedimentary rocks. Their fertility is variable, depending partly on factors which can only be adequately assessed on the spot ; the nutrient status of the soil as revealed by laboratory examination of a few samples alone is obviously insufficient to account for their productivity.

### Trinidad Soil Survey.

This has been continued by Dr. E. M. Chenery, Soil Surveyor on the staff of the Department of Agriculture, whose headquarters are at the College. Soil samples collected during the survey have been examined in the College laboratories, and the Head of the Department of Chemistry, acting in an advisory capacity, has been responsible for the further progress of this work. Detailed mapping has been completed by Dr. Chenery for the eastern part of Trinidad, and a provisional soil map, on the scale of about 6 inches to the mile, has been constructed by him. Lately, all the information available from any and every source referring to the soils of Trinidad has been utilised in constructing a land classification map on a scale of about 1 inch to the mile. This map should prove serviceable in the rehabilitation of cacao areas and the allocation of land settlements in Trinidad.

### Manurial Experiments.

A review of Dr. F. J. Pound's second year's report on the progress of manurial experiments on cacao in Trinidad and Tobago is included as part of this Annual Report. Some very interesting results are emerging from this work ; their possible general applicability to areas outside Trinidad furnishes sufficient reason for presenting a detailed summary of the main findings as a preface to this review.

### Ecological Investigations.

It is proposed to make a fresh attempt in 1939 (after an unavoidable lapse of nearly 3½ years' duration) to gain more information of the effect of changing environmental conditions on the growth and fruit-production of the cacao tree. As stated in last year's Annual Report, the next problem to be investigated is the cause of *fruit-shedding*, because this is believed to be the most important circumstance responsible for loss of crop, not only in Trinidad, but in many other countries where cacao is grown.

A general discussion of the factors controlling high production of cacao is presented in a special article published in *Tropical Agriculture*,* wherein attention is drawn to the interdependence between genetic (or internal) tendencies toward high yield, and actual expression or performance in the field. The value of an accurate assessment of these factors will become more and more evident when the behaviour of the recently-planted selected seedlings and clones has been thoroughly studied in Trinidad, although this may necessitate many years of careful observation. Subsequently, suitable adjustments of the environment may be necessary in order to achieve the full realisation of the high yielding capacity of each selected strain. Such adjustments involve a detailed knowledge of soil-types and manurial responses, as well as of atmospheric conditions in their effects on growth processes. The ecological studies should therefore be conducted concurrently with plant-genetical investigations, so that eventually the results may be combined and placed at the disposal of planters.

There are two alternative practical procedures available to the cacao grower ; one is to adjust the environment by suitable soil treatment (such as cultivation and manuring), and by proper horticultural practices (such as pruning), so that the selected types of cacao planted in that environment are able to realise their full yielding capacity. The other procedure is to choose a strain of cacao suitable to a particular set of

* Vol. XVI, No. 8, 1939.

environmental conditions. In the latter case, the cacao type may be selected for quality or for high yield, depending on commercial requirements and on the particular features of the environment, such as natural soil fertility. In either case, a complete understanding of the physiological ecology of the cacao plant is an essential prerequisite, although its full significance may not be appreciated until the botanists' selected cacao types have been long subjected to searching tests under varying field conditions.

### Cacao Biochemistry.

Biochemical investigations of cacao were begun towards the end of 1935 in response to a need for more information regarding the effects of environmental and genetic factors on the chemical composition of the bean and on those of its features vaguely expressed by the term "quality". Unfortunately, to make such investigations possible, all other chemical work had to be suspended. The manurial investigations and soil surveys were handed over to the officers of the Trinidad Department of Agriculture, and laboratory work on cacao soils became a charge on the Soils Department of the College.

After some three years of biochemical research, undertaken intermittently by three successive officers, the only real progress made has been the acquisition of knowledge and experience relating to laboratory methods for determining tannins, alkaloids and fat. The titles of the papers published in the Annual Reports for 1936, 1937 and 1938 indicate the scope of the work, and it is hoped soon to attempt an assessment of the contributions to quality made by the different chemical components of the cacao bean. The variation in fat content is particularly important because of the great commercial value of cacao fat. Any significant increase in fat production induced by suitably adjusting the environment or by planting a particular genetic strain of cacao would immediately command the attention of planters and manufacturers.

In the present report, Dr. E. C. Humphries presents four papers, two of which deal with aspects of the cacao fat problem, and two with the determination of alkaloids in the bean.

A summary of recent contributions to our knowledge of the biochemical components of the cacao bean has also been prepared by Dr. E. C. Humphries and Dr. H. F. Birch, and forms the last article of this Report.

## PART I.—CACAO SOIL INVESTIGATIONS.

## (1) SOME NIGERIAN CACAO SOILS.

By F. Hardy.

### (I) Introductory.

In further continuation of comparative laboratory studies of West African cacao soils, described in the last two Annual Reports (concerning Gold Coast Cacao Soils), a series of soil samples, 127 in number, representing 15 profiles, were collected in Nigeria by Mr. H. C. Doyne (Agricultural Chemist, Nigerian Department of Agriculture), and forwarded to the College in November, 1938, for laboratory examination by our routine methods. In addition, 35 soil samples, representing 4 profiles, previously collected by Mr. Doyne and submitted to us in October, 1931, were also examined. The detailed results have been sent to Mr. Doyne (March, 1939, and March, 1936), together with profile cards in which the laboratory data are depicted in colours for each layer of soil of each profile.

These results are not here reproduced, but averaged data for the surface 6-inch layer are brought together in a table, in which the data are compared with those for typical cacao soils occurring in the Gold Coast Colony and in Trinidad, Tobago and Grenada. In this table, the Nigerian cacao soils are arranged in the approximate order of their fertility as assessed by the laboratory results to which an arbitrary scheme of marking has been applied, exactly as in the case of the Gold Coast soils discussed in the last Annual Report.

Comparison between the fertility-status of the soils, as assessed by laboratory data, and their actual yielding capacities for cacao unfortunately is not possible in the case of the Nigerian cacao soils, because reliable yield data are not available. Average yields in Nigeria are stated to lie between 500 and 600 lb. dry cacao per acre, which is somewhat lower than the average figures for "good" and "medium" Gold Coast cacao soils listed in the last Annual Report, though approximately the same as that of average "good" cacao soil in the British West Indies.

It must again be strongly stressed that the nutrient factors of the soil are by no means the only factors affecting cacao production. Consideration of the discussion presented in another place * will indicate the views of the ecologist concerning the relative importance of different growth factors, among which the *water relations* of the soil and the atmosphere seem to be particularly important in the case of cacao. Assessment of the moisture factor is not included in the laboratory examination of soils, except in so far as its magnitude may be roughly gauged by sand contents, and index of texture values within the soil profile. The only way to procure a balanced assessment of the environment is to measure periodically as many of the factors that

* The Maximum Yield of Cacao. F. Hardy, *Tropical Agriculture*, XVI, 8, 1939.

## NIGERIAN CACAO SOILS.

SITES ARRANGED IN ORDER OF LABORATORY ASSESSMENT.

(*Summarised Data for Surface 6-ins Layer.*)

| No. | Site. | Sand Content. % | Index of Text. I.T. | Reaction pH. | Org. Matt. % | C/N Ratio. | Avail. Nutr. | Rate of Soln. | Avail. $P_2O_5$ | Avail. $K_2O$ | Soil Type.* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | mhos × $10^{-6}$ | | p.p.m. | | |
| 6. | Owena, Ondo ... ... | 67 | 13 | 7.0 | 4.9 | 10.4 | 227 | 148 | 138 | 230 | YGG |
| 12. | Ifo ... ... ... | 68 | 6 | 6.7 | 2.0 | 13.1 | 94 | 34 | (1387) | (472) | YSD |
| 2. | Ina, Ibadan .... ... | 71 | 9 | 6.9 | 2.2 | 9.5 | 138 | 78 | 103 | 193 | YGG |
| 7. | Owena, Ondo ... ... | 59 | 17 | 6.0 | 3.4 | 10.5 | 115 | 85 | 31 | 170 | YGG |
| 11. | Ilaro, Abeokuta ... ... | 40 | 19 | 6.9 | 2.5 | 10.8 | 128 | 60 | 68 | 113 | YSD |
| 16. | Moor, Ibadan ... ... | 89 | 0 | 6.4 | (0.9) | 10.4 | 64 | 37 | 52 | 142 | YGG |
| | Means (" Good ") ... ... | 66 | 11 | 6.6 | 3.0 | 10.8 | 128 | 74 | 65 | 141 | |
| 1. | Agia, Ibadan ... ... | 52 | 6 | 6.9 | 2.0 | 10.0 | 95 | 52 | 22 | 72 | YGG |
| 14. | Ife Rd., Ibadan ... ... | 71 | 4 | 6.3 | 1.4 | 14.6 | 63 | 34 | 6 | 119 | YGG |
| 15. | Ife Rd., Ibadan ... ... | 77 | 2 | 6.2 | 1.9 | 14.2 | 40 | 21 | 2 | 100 | YGG |
| 17. | Umuahia, Owerri ... ... | 68 | 4 | 4.1 | 2.0 | 15.2 | — | 11 | 22 | 80 | RSD |
| 9. | Bende, Owerri ... ... | 73 | 18 | 5.6 | 3.2 | 9.5 | 34 | 18 | 13 | 60 | YSD |
| | Means (" Medium ") ... ... | 57 | 7 | 5.8 | 2.1 | 12.7 | 58 | 27 | 13 | 86 | |
| 19. | Ogba, Benin ... ... | 80 | 0 | 4.1 | 2.0 | 13.7 | — | 10 | 18 | 85 | RBS |
| 10. | Ilaro, Abeokuta ... ... | 57 | 10 | 5.7 | 2.2 | 9.1 | 37 | 21 | 9 | 54 | YSD |
| 18. | Umuahia, Owerri ... ... | 77 | 0 | 4.5 | 1.3 | 12.6 | — | 14 | 19 | 60 | RSD |
| 3. | Benin ... ... ... | 87 | 1 | 5.8 | 1.3 | 11.2 | 30 | 14 | 13 | 62 | RBS |
| 5. | Ogba, Benin ... ... | 69 | 7 | 5.0 | 1.5 | 14.1 | 14 | 5 | 10 | 60 | RBS |
| 4. | Ogba, Benin ... ... | 78 | 2 | 4.7 | 1.4 | 13.2 | 41 | 5 | 8 | 83 | RBS |
| 13. | Agege, Colony ... ... | 86 | 9 | 6.0 | 1.3 | 14.5 | 25 | 10 | 5 | 38 | YSD |
| 8. | Nkwell, Onitsha ... ... | 77 | 2 | 5.1 | 1.3 | 12.9 | 22 | 5 | 7 | 57 | RSD |
| | Means (" Bad ") ... ... | 76 | 4 | 5.1 | 1.5 | 12.7 | 21 | 10 | 11 | 62 | |
| FOR COMPARISON. | | | | | | | | | | | |
| | (A) GOLD COAST CACAO SOILS. | | | | | | | | | | |
| | " Good " (Means of 13 cases) ... | 60 | 10 | 6.9 | 3.4 | 11.7 | 179 | 154 | 41 | 163 | |
| | " Medium " (Means of 13 cases) | 66 | 9 | 6.0 | 2.1 | 10.5 | 88 | 66 | 20 | 75 | |
| | " Bad " (Means of 14 cases) ... | 69 | 7 | 5.7 | 1.4 | 9.2 | 54 | 37 | 18 | 58 | |
| | (B) WEST INDIAN CACAO SOILS. | | | | | | | | | | |
| | *Trinidad.* | | | | | | | | | | |
| | Chocolate Soil ... ... | 36 | 43 | 7.2 | 6.0 | 9.6 | 216 | 175 | 343 | 309 | |
| | Brasso Sand ... ... | 60 | 18 | 6.2 | 2.5 | 7.8 | 100 | 87 | 39 | 267 | |
| | Moruga Sand ... ... | 62 | 15 | 5.7 | 2.9 | 10.5 | — | 35 | 19 | 128 | |
| | *Tobago.* | | | | | | | | | | |
| | Schist Soil ... ... | — | 35 | 6.7 | 4.8 | 10.2 | 180 | 177 | 82 | 87 | |
| | Diorite Soil ... ... | — | 39 | 6.4 | 4.7 | 10.2 | 175 | 160 | 30 | 94 | |
| | Alluvial Soil ... ... | — | 29 | 6.5 | 4.1 | 12.0 | 180 | 173 | 75 | 80 | |
| | *Grenada.* | | | | | | | | | | |
| | Basic Igneous ... ... | — | 40 | 6.9 | 3.7 | 10.7 | 135 | 117 | 85 | 380 | |
| | LIMITS OF ADEQUACY FOR SANDY SOILS. (Provisional Standards.) | | | | | | | | | | |
| | Minimum Values ... ... | — | 10 | 5.5 | 3.0 | 8.5 | — | — | 30 | 100 | |

*SOIL TYPES:

| *Red Earth.* | *Yellow Earth.* | |
|---|---|---|
| — | YGG | ...Granitic Gneiss. |
| RSD | YSD | ...Eocene Sediments. |
| RBS | — | ...Benin Sands. |

control plant growth as may readily be studied in the field and the laboratory ; this is the method used in Trinidad in our earlier investigations and its application is urged in researches on cacao now being undertaken in West Africa. Already in the Gold Coast, ecological researches of cacao along these lines have been initiated, and it is hoped that they may soon be started also in Nigeria.

The fact that the moisture-holding capacity of the soil "has vital bearing on the growth of cacao" is stressed in a recent article by Doyne and West (1) who urge the provision of adequate ground-shade for young cultivations, and as a means of preventing "dieback" in Nigerian cacao plantations.

**(II) Notes on Some Nigerian Cacao Soil Types.**

As far as the writer is aware, no comprehensive account of the soils of Nigeria has yet been published, although information concerning them is provided by articles written by H. C. Doyne, M. Greenwood, C. J. Lewin and others (*see* reference list).

According to Doyne and West (1)*, cacao cultivation in Nigeria is mainly confined to two types of soil, forming only a relatively small proportion of the climatic belt actually suitable to the growth of the crop. The successful cacao soil-types have been designated Type A (Oyo and Ondo Provinces), and Type F (Colony and Abeokuta Provinces). In addition, a few plantations also occur on Type H alluvial soil (Ogoja Province), and on Type L (Cameroons). The lands occupied by yet another soil Type D (Benin, Warri and Owerri Provinces), fail to produce cacao satisfactorily, except in the case of some of the more fertile representatives.

The series of soil samples submitted by Mr. Doyne include examples of Type A, Type F and Type D only. These are briefly described below. They all occur in South-western Nigeria, and all except one, within 70 miles of the coast.

*Type A.* (Oyo and Ondo Provinces). Derived from undifferentiated Archaean metamorphic rocks, chiefly *granitic gneiss* (YGG).

*Profile Nos.* 1 (Ajia), 2 (Ina), 14 (Ife 1), 15 (Ife 2), 16 (Moor) ;—Ibadan District.
6 (Owena 1), 7 (Owena 2) ;—Ondo District.

This distinctive soil-type has been described by Doyne and Watson (3) under the term "Ilepa", because of its characteristic white-and-red-mottled clay subsoil, known by that name to the Yoruba people. The surface soil is brown, humic, loose and sandy, containing over 60 per cent. of sand ; the subsurface soil usually contains abundant orange, red and black quartzose concretions (locally termed "laterite"), often forming an impervious pan ; the subsoil is red clay, grading downwards through mottled-red-and-white clay to white kaolinitic clay containing rotten granitic-gneiss brash. The unaltered parent rock is seldom reached at depths less than 10 feet. The annual rainfall is 50 to 60 inches, with a distinct dry season during December to March ; the temperature varies from 70° to 90° F. The natural vegetation is forest, replaced by secondary high bush (8).

The reaction of the Ilepa soil is increasingly acidic down the profile, though the acidity may decrease as the parent rock is approached (4). The profile is considered to be immature, and the occurrence of concretions of iron oxide and manganese peroxide around quartz particles in the subsurface soil is attributed to capillary rise of mineral matter in solution during dry seasons, and the precipitation of oxides when the solution reaches the less-acidic upper soil (3). In spite of the high degree of acidity, the soils are said to be rich in exchangeable lime (7).

The most typical Ilepa soils of the series submitted by Mr. Doyne are Profiles Nos. 14 and 15, (Ife Road, near Ibadan), and Profile 16 (Moor Plantation, Ibadan), although none of these is more acidic than pH 6.0. The two Owena profiles (Nos. 6 and 7), also placed in this class, consist of dark-brown sandy soil overlying red clay containing concretions and rock-brash below the 12-inch depth. The parent rock of the Owena soils appears to be biotite-hornblende granite. The downward increase in soil acidity is much greater than in the others of this type. Profile No. 6 (Owena 1) is alkaline in the first 12-inch layer, and has all the attributes of a nutrient-rich, highly humic soil.

All the examples examined of Type A comprise "good" and "medium" soils, according to the laboratory assessment given in the table ; they include no "bad" examples. The soil-type has tentatively been classed as Yellow Earth ; its features most nearly resemble those of the Cape Coast Granite Soil-type among the cacao soils of the Gold Coast.

*Type F.* (Colony and Abeokuta Provinces). Derived from undifferentiated *Tertiary sedimentary rocks* (YSD).

*Profile Nos.* 10 (Ilaro 1), 11 (Ilaro 2), 12 (Ifo) ;—Abeokuta District.
13 (Agege) ;—Colony District.

This soil-type appears to be very variable ; the parent rock is apparently either red grit, sand or sandy-clay with beds of kaolin (5). A belt of phosphate deposits is reported running 15 miles west from Ifo, and the soil here is stated to support kola and cacao trees that are "very notably above average size" (5). It is noteworthy that profile No. 12 (Ifo) contains exceptionally large amounts of available phosphate, being well above 1500 p.p.m. (Truog test) in the surface 12-inch layer ; this soil comes second on the list in fertility, and is stated to support healthy trees.

* We have not had the opportunity of seeing the original paper (2) in which appeared the summary given in this one.

Type F soils are described as dark-grey, heavy loams. They are sandier in the top 12 inches (I.T. 10), than in the lower layers which are quite clayey (I.T. 20), and contain abundant sepia shotty concretions, generally less than 3 mm. in diameter. Two examples (Nos. 11 and 13) show gley formation caused by waterlogging. The subsoils are typically highly acidic (pH 5.5 or less), and dull orange or reddish-sepia in colour. Organic penetration is deep (to the 3-foot depth), but mineral nutrients are deficient, except in the phosphatic kind of soil. Two of the samples fall within the "good" category, and two within the "bad".

*Type D.* (Benin and Owerri Provinces). Derived from Sedimentary rocks of middle Eocene or more recent age. This type may be subdivided into (i) Soils derived from *Benin Sands* (Post Middle Eocene, possibly Pleistocene), (ii) Soils derived from the *Lignite Group* (Post Middle Eocene), and (iii) Soils derived from the *Bende-Ameki Group* (Middle Eocene).

(i) *Benin Sands Sub-type.* (RBS.)

*Profile Nos.* 3 (Benin), 4 (Ogba 1), 5 (Ogba 2), 19 (Ogba 3) ;—Benin District.

This very distinctive soil-type is a bright-red (terra cotta) sandy soil of marked uniformity to great depth, and extremely highly acidic throughout the profile (pH 5.0 to 4.0). It is not considered suitable for cacao. Its high degree of acidity is an expression of its very low exchangeable base status

The Benin District occupies ridged country of elevation 250 feet, having annual rainfall around 75 inches and no marked dry season (7). Cacao plantations here occur only sporadically.

Humic penetration in this soil-type is relatively shallow, usually less than 18 inches, and mineral nutrients are very deficient. Each of the four representatives belongs to the "bad" category. No distinctive differences could be detected by laboratory methods between Profiles No. 4 and No. 5, which represent soils in the vicinity of a "poor" and a "good" cacao tree respectively, although each is very highly acidic and very deficient in available phosphate and potash.

(ii) *Lignitic Clay Soil-type.* (RSD).
*Profile Nos.* 8 (Nkwell):—Onitsha District.
17 (Umuahia 1), 18 (Umuahia 2):—Owerri District.

The first of these (No. 8) is described as derived from a "sedimentary ironstone formation", which conforms with the statement that the Lignite Group often contains ferruginous material (6). Nevertheless, this example must be regarded as exceptional. It is a bright red, very highly acidic sandy soil having a very low degree of fertility (bottom of the list). Its location is stated to be "outside the cacao area".

The two Umuahia soils (Umdike Farm ; Nos. 17 and 18), were taken from a site on the fringe of the Oil Palm belt, altitude 500 feet, annual rainfall nearly 100 inches, with dry season extending from November to March. The soils vary from brownish-yellow clays to red sandy-loams, and are very highly acidic in reaction (pH 4.5 to 4.0).

The parent rock of the Lignitic Clay soils is commonly a massive "pale-coloured clay, sometimes mottled or stained throughout by "ferruginous material, and sometimes interbedded "with fine sandy-clay" (6). Lignite is usually associated with a greenish-brown facies, but it is also interbedded with the pale clays (6).

The cacao trees at Umdike Farm dry out badly during the rainless season, and become subject to termite attack (7, 8, 9).

(iii) *Bende-Ameki Sub-type.* (YSD.)

*Profile No.* 9 (Bende):—Owerri District.

This single example is stated to be derived from a "yellow sandstone" which is highly micaceous. It is a very sandy, black, humic soil (73 per cent. sand), highly acidic in reaction (pH 5.0), deficient in mineral nutrients, and of medium fertility.

## (III) Conclusions.

It is evident from these brief notes and the paucity of the literature on Nigerian cacao soils that much further research is needed into the ecological relationships of the cacao tree in that country before a true assessment can be made of the contribution to yields that is afforded by soil nutrients. Factors other than chemical composition of the soil should be studied in detail, preferably by means of periodic measurement of soil moisture contents and atmospheric conditions as indicated by meteorological instruments.

## (IV) References.

(1) Some Aspects of the Soil Requirements of Cacao in Nigeria. H. C. Doyne and J. West ; *West African Agricultural Conference*, 1938, pp. 19-21.
(2) Soil-types and Manurial Experiments in Nigeria. *West African Agricultural Conference*, 1938 (Not seen).
(3) Soil Formation in Southern Nigeria (The Ilepa Profile). H. C. Doyne and W. A. Watson ; *J. Agr. Sci.*, 1933, 23, pp. 208-215.
(4) Studies in Tropical Soils : Increase of Acidity with Depth. H. C. Doyne ; *J. Agr. Sci.*, 1935, 25, pp. 192-197.
(5) Reconnaissance Survey of Parts of Abeokuta and Oyo Provinces. W. Russ ; *Annual Rept. for* 1933, *Geol. Surv. Dept., Nigeria*, 1934, pp. 43-50.
(6) Water Supply in Benin Province. C. M. Tattam ; *Annual Rept. for* 1933, *Geol. Surv. Dept., Nigeria*, 1934, pp. 10-16.
(7) Some Cacao Soils of the Eastern Plains in Nigeria. M. Greenwood ; *Tenth Annual Bul., Ag. Dept. Nigeria*, 1931, pp. 36-49.
(8) The Maintenance of Soil Fertility in Southern Nigeria. C. J. Lewin ; *Third Spec. Bul., Ag. Dept. Nigeria*, 1931, pp. 1-43.
(9) Umuahia Agricultural Station—Review of Farm Work, 1923-37. F. E. Buckley ; *West African Agricultural Conference*, 1938, pp. 61-74.

# (2) MANURIAL EXPERIMENTS ON CACAO IN TRINIDAD—SUMMARY OF RESULTS FOR 1938.

**(With an Introductory, indicating the General Applicability of some of the Findings and Conclusions.)**

By F. Hardy.

## Introductory.

In continuation of last year's account, the following review of Dr. F. J. Pound's report for the year ending 31st August, 1938, (123 typescript pages), submitted to the Cacao Subsidy Board, is here presented for the purpose of indicating the magnitude of the yield-responses obtained by manuring cacao growing on the diverse types of soil that occur in Trinidad and Tobago. As in last year's summary, the main results only will be considered, and all yield data will be expressed as pounds weight of dry cacao per acre of land.

Since Dr. Pound took charge of cacao manurial experiments in January, 1936, the number of Latin Square experiments (Type A) has been increased from 9 to 45, distributed among 8 soil-types, all but one of which are derived from Sedimentary Formations (sands, silts and clays). In addition, a large number of less elaborated experiments (not all of which are manurial) have been initiated, and these include some 200 acre-block (Type B) experiments, together with special cultivation, pruning, rehabilitation and propagation experiments. Although the majority have produced only two years' yield data, certain striking indications have emerged, which are thought to be sufficiently important to warrant publication. It is hoped that the information may prove useful to investigators of cacao problems outside Trinidad.

The writer tenders his thanks to Dr. Pound and to the Director of Agriculture for permission to issue these results in their present form.

### Summary of Main Findings and Conclusions.

(1) Prior to 1930, it had been found in Trinidad that applications of *pen manure* were beneficial to cacao, and the conclusion was naturally drawn that the component responsible for the increased yields was *nitrogen*. Ammonium sulphate, used as substitute, failed to give commensurate responses, however, sometimes actually depressing the yields. Subsequent experiments proved that the valuable component of pen manure is generally not nitrogen, but *potash*, though the cellulosic and other carbohydrate components and occasionally phosphate, may play a part in causing increased yields of cacao on different types of soil. These results apply to cacao growing under shade; nitrogen does not necessarily depress yields of *unshaded* cacao, and small dressings of ammonium sulphate have been found to be beneficial to cacao deprived of shade, or heavily pruned, and to cacao growing on certain poor, sandy, shallow soils. Apparently, Immortel trees as normally distributed in cacao fields in Trinidad and Tobago, are capable of providing, by the fall of leaves, twigs, flowers and fruits, sufficient nitrogen for the needs of the cacao crop. Their symbiotic association with root nodule-inhabiting, nitrogen-fixing micro-organisms enables them to assimilate nitrogen from the atmosphere, and so to enhance the soil's supply of nitrogen.

(2) During the progress of the manurial experiments, it has become evident that *profits from manuring* cacao depend, not only on (i) the price of cacao, but also (ii) on the nutrient status of the soil, and (iii) on the condition of the trees. While large percentage increases in yield may be obtained with some soil-types by appropriate manuring, the operation is rarely profitable at present low prices of cacao. On the other hand, if the gross nutrient deficiencies of phosphate and potash in the soil are first made good by the addition of relatively large amounts of artificial manures, the residual effects in subsequent years may be appreciable, even if no more manure is added. Preferably, the initial large dressings should be followed by small annual dressings in order to maintain the nutrient status of the soil at a high level; in many instances, the net result over a series of years may then be profitable. In any case, it may be unprofitable to manure a field of cacao which is not in a suitable condition to respond to the treatment, by reason of the fact that a large proportion of the trees have been broken by falling shade trees, or damaged by insect and fungus attack.

(3) A programme of heavy capital-expenditure manuring should therefore be preceded by general cultural treatment, involving thorough draining, where necessary, round-ridging, and pruning with a view to rejuvenating dormant trees.

> In an experiment in Tobago, cacao trees were so heavily pruned before manuring that they gave only a small crop during the following year, namely, about 170 lb. of dry cacao per acre. In the next year, the rejuvenated trees responded so well to the manures that the yield increased to over 1,000 lb. per acre.

(4) It has been demonstrated in these experiments that *phosphatic manures* may produce very large immediate increases in yield (of the order of 100 per cent.) when applied to certain phosphate deficient clay soils growing cacao in Trinidad, and that marked residual effects usually ensue when the applications are subsequently withheld. Superphosphate and other soluble phosphatic manures have given the quickest responses, but ground rock phosphate, while much slower acting, eventually may make good

the gross phosphate deficiencies, and prove to be equally effective in the long run. The most profitable responses have been given by relatively small dressings of phosphatic manures, but larger dressings in due course may prove profitable, if the cost is spread over a series of years.

(5) Similar results have been obtained for *potash manures* applied to potash-deficient sandy cacao soils, but here the magnitude of the annual dressings must not be excessive, otherwise marked *yield depression* may follow. This optimal relationship of potash manure to cacao yields appears to be unique in the nutrition of fruit trees, where the application of increasing amounts of manure is generally accompanied by corresponding increases in yield.

> For example, in manuring grapefruit, the dressing of mixed artificial manures recommended for application to trees transplanted from the nursery is usually augmented by regular annual increments, so that, at 20 years old, the dressing may be as much as 60 lb. of mixed salts per tree. This difference in manurial requirements between cacao and grapefruit may perhaps be attributed to the fact that, in the case of cacao, the yield- increase concerns the seeds, whereas in the case of grapefruit, it concerns the juicy pulp, the seeds being unwanted. Furthermore, grapefruit trees are planted much wider apart (say 30 by 30 ft.), and their root-system is probably more widespread than that of cacao, necessitating increased amounts of manure.

(6) The *best time for applying* manures to cacao in Trinidad, as indicated by certain of the manurial experiments, is five or six months *before the main crop is reaped*, that is, at the time when the young fruits are setting, namely, October in eastern Trinidad, and May in western Trinidad. This suggests the possible significance of mineral nutrition in the prevention of fruit-shedding of cacao, which is believed by many planters to be the chief cause of low yields in certain fields.

(7) The results of those manurial experiments whose quantities were based on data derived by chemical laboratory tests applied to representative soil samples collected within the area of the experiment fully demonstrate the value and reliability of the tests as a means of deciding initial deficiencies of available phosphate and available potash. Such chemical tests should preferably precede the designing and the laying down of manurial experiments on areas where the soil is liable to great lateral variation in nutrient status.

The following lower limits of adequacy appear to apply to cacao soils in Trinidad and Tobago, for the surface 6-inch layer.

(A) *Available phosphate* (Truog's method ; $P_2O_5$ parts per million of air-dry soil).

(i) *Clay soils* (Index of Texture above 25) ; 50 p.p.m.

(ii) *Sandy soils* (Index of Texture below 25 ; 30 p.p.m.

(B) *Available potash* (Exchangeable potash, extractable by N/2 acetic acid ; $K_2O$ parts per million of air-dry soil).

(i) *Clay soils* (Index of Texture above 25) ; 175 p.p.m.

(ii) *Sandy soils* (Index of Texture below 25) ; 100 p.p.m.

(8) A remarkable response to an application of cane-sugar (3000 lb. per acre) has been recorded for acidic alluvial sandy soil at River Estate, where a yield increase of 24 per cent. was obtained in one year. This result is regarded as confirming the positive relationship between high yield and high carbon-nitrogen ratio that has previously been demonstrated for West Indian cacao soils.

(9) The *natural yields* of the cacao soils of Trinidad and Tobago vary from less than 100 lb. of dry cacao per acre to over 1,200 lb. per acre (for example, at Roxborough in Tobago). Manurial responses likewise vary from negligible amounts to over 100 per cent. of the natural yield, depending on many factors, some of which are discussed in an article on the Ecology of Cacao (*Tropical Agriculture*, XVI, 8, 1939). The highest yield recorded by Dr. Pound is 2,088 lb. acre at Roxborough, Tobago, for an experimental plot liberally treated with pen manure and artificial manures.

(10) An experiment designed to evaluate the *effects on yield of draining* alluvial soil growing cacao under different degrees of shade gave striking results over a period of eight years, that are believed to demonstrate the complementary operation of drains and of shade trees. Under shade of highest intensity, the drains were the least effective, but under shade of low intensity, the drains placed at closest distance apart proved to be the most effective.

### Results of the Manurial Experiments.

#### (1) Acidic Alluvial Clays of River Vegas and Basins.

(A) Northern River Vegas :

This is the soil-type on which the first phosphate manurial trials were laid down (at Las Hermanas Estate) in 1932 by J. A. McDonald as a direct outcome of an ecological comparison between a "good" plot and a "bad". This soil was shown to be deficient in available phosphate by chemical tests, though its content of available potash was apparently adequate. The natural yield in recent years has been low, ranging from 200 to 260 lb. dry cacao per acre. In the first experiment (A2)* *superphosphate* applied annually for four successive years gave up to 73 per cent.

*The symbols in brackets are the code numbers of the experiments. Latin Square experiments are labelled "A".

increase in yield, but during the two years following, when no manure was applied, the residual effect diminished to zero. Only the smallest dressing (1 lb. per tree) proved profitable at current low prices for dry cacao (5c. lb. in the field), and this gave a financial return of 42 per cent. on money expended on the manure.

In another Las Hermanas experiment (A3), annual applications of *ground rock phosphate* were found not to be so effective as superphosphate during the first three years, but last year, rock phosphate gave the greater yield increase, and proved to be equally efficacious over the whole period of the experiment. So far, however, none of the treatments has been profitable at current low prices of cacao. In yet another experiment (A4), these two phosphatic manures were tested against *ammonium phosphate* ("Nicifos I") and *double superphosphate*, both very soluble and highly concentrated manures. During the three years of this trial, the three soluble phosphates gave similar yield increases (between 30 and 40 per cent.) and each of them gave higher yields than the insoluble ground rock phosphate (13 per cent.), but the dressings used (3 lb. per tree) were too expensive to yield any profits. Potash manure conferred no additional benefit, neither in this nor in the previous experiment.

*Liming Experiments.*—Even when treated with the optimum dressing of phosphatic manure, the yields of cacao on this soil-type are always unsatisfactory, seldom exceeding 500 lb. per acre. It has been suggested therefore that the soil's structure is at fault, for example, its high degree of compaction may be causing lack of aeration. Accordingly, a new experiment (A29) was laid down in 1936 to test the effect of liming as a means of granulating the soil, and so improving its fertility. The dressings were 5, 10 and 20 tons of ground limestone per acre. The records are insufficient to warrant conclusions at this stage, but the liming appears to be exerting some beneficial effect after two years. It will take a much longer time for the limestone to penetrate this compact soil; the land was not forked nor cultivated, and it is well known that lime "only acts where it touches".

In another liming experiment (A28), the effects of pen manure, potassium chloride and basic slag were tested on acidic alluvial clay that had been limed the year before (in 1936) at the rate of 20 tons ground limestone per acre. So far, the only response is from pen manure, but more time should be allowed, as in the last experiment, for the limestone and the slow-acting basic slag to penetrate into the soil.

(B) Guapo Flat.

An experiment (A32, 33) laid down in 1936 on a phosphate and potash-deficient, highly acidic, heavy alluvial clay, occurring at Guapo in South-west Trinidad, showed no significant response to superphosphate, ground rock phosphate, and potassium sulphate, nor to liming and forking. In 1937, another experiment (A44) was initiated on the same site, in which the phosphatic manures employed were superphosphate, basic slag and ammonium phosphate ("Nicifos 4"). The first year's results do show some effect from "Nicifos" (40 per cent. increase in yield), and from basic slag (30 per cent.), so that response to the right kind of phosphate manure may be expected in future.

(C) Sangre Grande Flat.

This area, occupied by a highly acidic, nutrient-deficient, clay-silt soil, is subject to periodic flooding during rainy seasons. It also regularly suffers severe attacks by cacao Thrips, as well as recently by a disease affecting the Immortel shade trees.

A phosphate experiment (A17) was laid down on this soil in 1936, but Thrips obliterated the first crop, so that only the second year's returns were available. These demonstrated that 2 lb. superphosphate per tree caused an 18 per cent. increase in yield when applied in October (during the "Indian Summer"), but only two per cent. when applied in May before the rains, a rather unexpected result.

Another manurial experiment (A30, 31) was laid down at the same time to test superphosphate and potassium sulphate, singly and combined both on soil that had been limed and soil that had not. No response was obtained in the first year, because of Thrips damage, but, in the second year, superphosphate plus potash gave an increase of 25 per cent., the actual yield of cacao being nearly 500 lb. per acre. A similar experiment (A25), on an area not liable to Thrips, also showed benefit from potash manure, but not from phosphate, although a third experiment (A26), in which comparison was made between superposphate and ground rock phosphate, showed a decided benefit from each, of the order of 53 and 30 per cent. Finally, a limestone experiment (A27) showed an increased yield of 23 per cent. from five tons of ground limestone per acre in the second year.

A most interesting experiment (A41) on the Sangre Grande acidic clay-silt strikingly demonstrated the great benefits of combined cultivation, spraying against Thrips, and manuring. In the first year, draining, spraying with Bordeaux Mixture, and manuring with pen manure (20 tons per acre) and artificial manures (superphosphate, 3 lb. per tree; potassium sulphate, 2 lb. per tree), with liming (ground limestone 7 tons per acre), gave an increase in yield of 164 per cent., *i.e.* from 228 lb. to 603 lb. per acre.

(D) Fishing Pond Area.

This lies nearer the eastern sea-coast than the Sangre Grande Flat, and comprises sandier soil richer in mineral nutrients. A phosphate experiment (A38, 39) laid down here in 1937 gave bigger yield-increments with basic slag and ammonium phosphate ("Nicifos 4") than

with superphosphate or ground rock phosphate, the last-named being the least effective of all. Potash manure so far has given no significant additional benefit.

(II) Acidic Alluvial Sands and Silts of River Vegas of Northern Range Valleys.

The experiments on this soil-type are mostly located at River Estate in the Diego Martin Valley, west of Port-of-Spain, where the rainfall is relatively low (65 ins.), though well distributed throughout the year. The results of nine (9) manurial experiments (A1; A8 to 14; A18 to 20) were considered in last year's report. The current year's results add little to the information beyond confirming it. They amply demonstrate the striking effects of *potash manures*, which have on some occasions nearly doubled the yields when applied in relatively small dressings of, say, 2 lb. per tree (A14). The natural yields obtained on this soil vary between 500 and 600 lb. of dry cacao per acre, and yields up to 1,100 lb. per acre have regularly been obtained by simple potash manuring, and over 1,500 lb. in one case by a combination of potash and phosphate manures (A11). The response is somewhat erratic, depending mainly on the initial content of available potash and phosphate.

Inorganic *nitrogenous manures* (for example, ammonium sulphate) definitely depressed the yield, and diminished the response due to potash and phosphate manuring (A11, 12). Potassium chloride gave slightly greater increases than the more expensive sulphate (A14), and the suspected deleterious effect of chloride has not yet become manifest.

In an experiment (A1) designed to assess the *residual* effect of potash manuring on plots that had been treated for three successive years and then not treated for two more years, a marked increase in yield was still evident. The total cost of producing one bag (165 lb.) increase at the current price of potash manure was reduced from $6.67 to $5.62 through this residual effect. The total profit gained per acre was $4.12, with dry cacao worth 5c. per pound in the field.

A new experiment (A43) designed to test the effects of increasing doses of potash manure (3, 4 and 5 lb. per tree),on plots that had received a basal dressing of pen manure and ammonium phosphate, showed no superiority of the largest potash dressing over the smallest. In fact, evidence has been adduced from this and other similar experiments on sandy soil of the River Estate kind, which proves that an actual depression in yield may follow the application of over-doses of potash manure, or of normal dressings applied to soils already adequately supplied with potash (say, over 150 p.p.m. exchangeable potash in the surface six-inch layer).

*Pen Manure Experiments; Trenching.*

Burying pen manure and vegetable waste in trenches dug between the rows of cacao trees has been a regular practice at River Estate for many years. Several experiments designed to test the efficacy of this method (costing $40 per acre) as compared with surface dressing with pen manure and mulch (costing $32, or $38 per acre if forked in), with and without the addition of artificial manures and lime, have been laid down in recent years. One of these (A19), initiated in 1936, showed that organic manure lightly forked into the soil gave an average annual increase over two years of 70 per cent. on a natural yield of 440 lb. dry cacao per acre, while surface-spread organic manure gave 53 per cent. increase, and trenching, 46 per cent. increase. So far, the treatments have been unprofitable, but it is believed that next year there will be a definite balance in favour of mulching, even with cacao valued at 5c. per pound in the field.

The addition of potash manure (3 lb. per tree) to plots that had previously been trenched (A8, 9, 10) gave an additional increase of about 20 per cent. A comparison (A18) of potash manure with phosphate on a recently trenched field of cacao liable to Thrips showed no benefit from the phosphate, but an increase of 34 per cent. from potash manure (2 lb. per tree), over a yield of 590 lb. per acre given by trenching. A similar experiment (A20), laid down in 1936, gave a 53 per cent. increase over a natural yield of 540 lb. per acre, from potash and phosphate manures (2 lb. per tree each of potassium sulphate and superphosphate) applied over trenches. Artificial manures alone gave a 44 per cent. increase, and trenching alone, a 21 per cent. increase. Liming in this, and other, experiments showed no benefit on the sandy alluvial soils at River Estate.

*C/N Ratio Experiment.*

In order to test the effect of high carbon-nitrogen ratio organic matter (believed to favour high production in West Indian cacao soils), an experiment (A40) with potash and basic slag was laid down in 1937, with a half-plot treatment of *cane-sugar*, applied at the rate of 3,000 lb. per acre. The first year's results were very striking; they showed a 24 per cent. increase in yield due to the sugar alone, over a natural yield of nearly 640 lb. of dry cacao per acre. Potash showed some added benefit, but basic slag showed none. The sugar treatment is not practicable, but perhaps minced canes or molasses might give similar yield increases, and they should be tested in a future experiment. No attempt has been made to trace the cause of the yield response nor the mode of operation of sugar used as cacao manure.

A special 3-acre demonstration to test the combined effects of draining, round-ridging, mulching and manuring with potash (2 lb. potassium sulphate per tree) and phosphate (2 lb. superphosphate per tree), was laid down in 1937 on a cacao field (No. 10) at River Estate which was being rehabilitated. The first year's results showed little or no response to draining and round-ridging alone, but the addition of mulch and artificial manures gave an increase

of 113 per cent. of dry cacao per acre, or of 76 per cent. per 300 bearing trees, most of the contribution (69 and 84 per cent.) being made by the artificial manures.

(III) Neutral Black Clays: Princes Town Marl Type.

This interesting " rendzina " soil is known from chemical tests to be particularly deficient in available phosphate, though it is adequately supplied with available potash. It is a stiff black clay, neutral to slightly acidic in the surface layers, but alkaline and calcareous in the parent yellow clay. An experiment (A5, 6) laid down in 1936, in which equivalent dressings of superphosphate, ammonium phosphate ("Nicifos I"), double superphosphate and ground rock phosphate were compared, with a potash treatment superposed, showed yield increases only for superphosphate (36 per cent.) and for double superphosphate (24 per cent.). The dressing of superphosphate (3 lb. per tree) was thought to be excessive; yield increments from this treatment decreased in successive years after a 78 per cent. average increase in the first year. Only the superphosphate treatment so far has shown a profit ($6.55 per acre) in this experiment. A dressing of 2 lb. per tree might have been more economical. Potash slightly depressed the yields.

*Gypsum Effect.*

A special experiment (A7) designed to test the effect of gypsum (calcium sulphate) contained in superphosphate was initiated in 1935 on black clay soil. Three years' results show that gypsum (6 lb. per tree) applied alone, depressed the yield by 5 per cent.; superphosphate (4 lb.) plus gypsum, *increased* it by 10 per cent.; superphosphate alone increased it by 20 per cent., and double superphosphate (which contains no gypsum) (2 lb.) by 27 per cent. The gypsum effect is evidently small, though significantly depressing, considering the rather large amounts used. The soil is slightly alkaline, however, and greater depressing effects might occur on acidic clay soils, which should be tested in order to explain the well-known lack of response to phosphate manures which naturally-gypseous acidic clay soils (for example, Brasso Clay Soil) have shown in manurial experiments.

Other manurial experiments (A36; C1) that have been running only one year, substantiate the general experience with cacao manuring on this distinctive soil-type, namely, (*i*) the great benefits accruing from superphosphate manuring (60 to 100 per cent. increases in yield over a natural yield of 450 to 500 lb. of dry cacao per acre), (*ii*) optimum results from 500 lb. per acre superphosphate applications (say, 1½ lb. per tree), (*iii*) the superiority of soluble phosphatic manures over insoluble ground rock phosphate, (*iv*) lack of response to potash manure and to pen manure (though its action may be delayed), and (*v*) lack of response to liming and to forking, perhaps implying satisfactory physical soil conditions.

(IV) Undifferentiated Calcareous Clays.

Soils of this type, as indicated by Dr. Pound, occur in various parts of Trinidad, and will eventually be classified when the soil survey now being prosecuted by Dr. E. M. Chenery is completed. Manurial experiments on calcareous clays (A21) at Palmiste (South of San Fernando), Manzanilla (A16), Tamana, Guaracara, &c., laid down in 1936, have furnished data for two years, and useful conclusions may now be drawn from them. Compared with black clay soils derived from marl, these ochre-yellow clays require careful working if they are to respond to manurial treatments. Pen manure appears to give better returns on the calcareous clays; its cost is not recoverable in the first two years at the present low price of cacao, but the effects are believed to extend over 6 to 8 years. Phosphatic and potash manures confer definite benefits, the former being the more effective, but in two years no application was paid for by the profit from the increased yield. The best time for phosphate applications in the Manzanilla District of East Trinidad was found in an experiment to be October.

(V) Brasso Clay.

This characteristic soil stretches in a belt across Trinidad, north of the Central Range divide, in the direction W.S.W. to E.N.E. It probably includes several soil-types not yet described nor accurately delineated. In general, a gypseous land-slip type occupies cacao lands in the west of the belt (Montserrat District), and a less gypseous non-slip type, containing more calcium carbonate in the upper 3-foot layer, occurs in the east (Manzanilla District). The fertility of these soils varies greatly, but the gypseous type, supporting cacao over 20 years old, has exhibited declining yields during recent years. This type so far has failed to respond to phosphatic and potash manuring.

An experiment (A34) was laid down in 1936 on acidic Brasso Clay (containing 79 p.p.m. available phosphate but only 114 p.p.m. available potash) to test the effect of lime (5 tons ac. ground limestone), ground rock phosphate (2 lb. per tree) and increasing amounts of potassium chloride (2, 4, 6 lb. per tree). The first year's yields were very poor (less than 300 lb. per acre), but the second year's yields were good (523 lb. per acre), though the increasing doses of potash proportionately depressed the yield below that given by the 2 lb. treatment (552 lb. per acre).

Other demonstration experiments on Brasso Clay, or alluvium derived from it, involved ground limestone, ground rock phosphate or superphosphate, potassium chloride and manganese sulphate (25 lb. ac.). Increased yields of the order of 30 per cent. were obtained, but no definite conclusions can be drawn apart from noting the benefits which both sorts of phosphatic manure and manganese sulphate imparted, and the absence of depressing effect from the potash. The dressing of manganese salt may have been too small. (This treatment was presumably

included partly to test a suggestion put forward by J. A. McDonald (*Fifth Annual Report*, p. 46), that manganese deficiency may be a factor contributing to low yields of cacao growing in "bad" Brasso Clay Soil.)

(VI) Moruga Sands and Silts.

These cacao soils, occupying considerable acreages in South Central Trinidad around Penal, Siparia and Moruga, show variable though usually low contents of available phosphate and potash by chemical tests. An experiment (A37) laid down on soil very deficient in both these nutrients gave very large increases in the first year after the application of potash and phosphatic manures. A basal dressing of 2 lb. potassium sulphate per tree over the experimental area increased the yield *four-fold* above that of the surrounding areas (*i.e.* from an average of 100 lb. to over 430 lb. acre), while super-phosphate, 4 lb. per tree, in addition, further raised the yield to 660 lb., an additional increase of 52 per cent. Ammonium phosphate ("Nicifos 4") exerted a lesser effect, but it increased the yield by an additional 43 per cent., while basic slag (an insoluble manure) only increased it by 5 per cent. In their manurial responses, the Moruga sandy soils somewhat resemble the Northern Range alluvial sands, such as the River Estate soils.

(VII) Manzanilla Loams (Marper Farm, Govt. Experiment Station).

This area of 100 acres of very mixed soils, mostly silty, is the site of an elaborate series of manurial experiments laid down in 1930 (see *Trop. Agric.*, 1936, 13, p. 233). There are eight experiments in all, designed to test severally, lime, nitrogen, phosphate, potash, nitrogen plus phosphate, nitrogen plus potash, phosphate plus potash, and nitrogen, plus phosphate plus potash (*i.e.* Ca, N, P, K, N+P, N+K, P+K, N+P+K). Each manure, except lime, was applied every other year in single, double and triple doses. In 1936, half-plot treatments of extra potash were applied to the lime and the nitrogen plots, and half-plot treatments of lime to the mineral manure plots not dressed with lime. The cacao trees were pruned periodically to remove "Witches' brooms", and sprayed with Bordeaux Mixture to destroy Thrips. Adequate surface drains were provided. The area produced a record crop in 1937-38, several of the manured plots yielding over 1,200 lb. per acre of dry cacao, while surrounding estates were yielding at less than 150 lb. per acre.

Without going into details, the following facts have been established:—(*i*) liming has been beneficial, within the eight years of the experiment, when applied to acidic soils away from the limestone outcrops that occur in part of the area; (*ii*) potash manure has given profitable returns when applied at the low rate of 100 lb. of potassium sulphate per acre; there was no advantage in applying bigger doses; (*iii*) phosphatic manures have profitably augmented the potash manure when applied at the rate of 325 lb. superphosphate per acre; (*iv*) nitrogenous manure has depressed the yields or diminished the beneficial effects obtained from potash and phosphatic manures; (*v*) witchbroom disease has been economically controlled in this area, and the yields have been maintained, in spite of its prevalence, at 500 lb. dry cacao per acre.

An important outcome of this Marper experiment is the proof that potash and phosphatic manures show mutual relationship in the nutrition of cacao, the uptake of the one apparently inducing the uptake of the other, although doses larger than the double unit dose were not only unprofitable but actually wasteful.

(VIII) Soils of the Toco District of Trinidad.

This area, situated in the north-east of Trinidad, contains three main soil-types that have not yet been fully described nor mapped in the field. They have tentatively been named (1) Calcareous-Schist Soil, (2) Quartz-Schist Soil, and (3) Igneous Rock Soil, after the kinds of parent rocks that have given rise to them.

(1) *Calcareous-Schist Soil.*

This naturally supports fairly good crops of cacao, but it is characteristically very deficient in available potash, though its phosphate status is usually satisfactory. Cacao commonly exhibits pronounced marginal leaf-scorch on this soil-type; this affection is found frequently to be associated with a potash-deficient soil (*Sixth Annual Report*, pp. 13-24).

Three five-acre block demonstration experiments were laid down on acidic Calcareous-Schist Clay Soil in 1936. In two of these, the treatments were, lime (5 tons ground limestone per acre), superphosphate (1½ or 2 lb. per tree), potassium sulphate (1 or 2 lb. per tree), the amounts varying with the content of available phosphate and potash as determined by chemical analysis. In each case, an average increase in yield greater than 40 per cent. was obtained over the two-year period on soil whose natural yield varied from 220 to 370 lb. dry cacao per acre.

The third experiment (potash and ground rock phosphate) has not yet given any significant responses from cacao suffering severe leaf-scorch.

(2) *Quartz-Schist Soil.*

This soil nowadays supports only poor crops of cacao. It contains apparently adequate amounts of available potash, though variable amounts of available phosphate. It regularly suffers severe Thrips damage.

Two demonstration experiments laid down in 1936 on this soil-type involved potassium chloride and ground rock phosphate. In one of them, fair increments in yield have been obtained, but it is believed that the insoluble phosphatic manure has not yet acted fully, while in the other, on a high-yielding site, no increased yields have yet been obtained.

(3) *Ingneous Rock Soil.*

This (for Trinidad) unique soil has been derived from granodiorite or andesite, and is believed to be a quite fertile soil. Cacao growing on it shows only sparse leaf-scorch, and suffers but little Thrips damage.

No Latin Square manurial experiment has yet been laid down on this igneous soil at Toco.

NOTE.—In 1937, a special experiment was laid out on each of these three main Toco soil-types, to test ammonium phosphate ("Nicifos I") and potassium sulphate on cacao trees that had been systematically pruned. The first year's results showed big responses of cacao growing on *Calcareous-Schist Soil,* of the order of 120 per cent., from a dressing of 3 lb. potash manure and 1 lb. "Nicifos" per tree, with poor-bearing cacao yielding at the rate of only 192 lb. per acre in the control plot. The response on *Quartz-Schist Soil* was not so large, *i.e.* 76 per cent., for a dressing of 1 lb. per tree of each of the two manures, for soil yielding 174 lb. per acre. The *Igneous Rock Soil* gave no increased yield from the manuring.

(IX) SOILS OF TOBAGO.

Dr. Pound subdivides these into (1) Sandy-Loams and Gravels of the King's Bay District and Northwards, (2) Heavy Alluvial Loams of the Richmond District, and (3) Gravelly-Loams of the Roxborough District. These soil-types are all derived from igneous rock (epidiorite), similar to that which occurs at Sans Souci, Toco. Type No. 1 may include (north of King's Bay) soils derived from quartz or talc schists (see *Soils of Tobago,* 1931, I.C.T.A.). Each of the types is deficient in available potash, and marginal leaf-scorch is conspicuous in many of the cacao fields. Available phosphate content is variable. The soils are usually slightly acidic (pH 6.2 to 6.8) and range in texture from sands to clay-loams.

(1) *King's Bay Sandy-Loam and Gravel.*

An experiment (A23) laid down in 1936, designed to test the effects of potash and phosphatic manures, gave a 40 per cent. increase in yield in the second year, over a natural yield of 850 lb. dry cacao per acre (*i.e.* nearly 1200 lb. ac.) for a mixture of the two manures, applied at the rate of 1½ and 3 lb. per tree respectively. Potash manure alone gave an increase of 25 per cent., and phosphate alone, 20 per cent. An acre demonstration experiment gave similar results for a mixture of potash manure and ground rock phosphate.

(2) *Richmond Alluvial Loam.*

A similar experiment (A24) was laid down on this soil in 1936 to test potash and phosphatic manures with and without lime (5 tons acre ground limestone). The response to liming was found to be negligible, and only potash manure (2½ lb. potassium sulphate per tree) gave a significant increase in yield, of 21 per cent. for cacao yielding naturally at the rate of 590 lb. per acre.

(3) *Roxborough Gravelly-Loam.*

This variable nutrient-deficient shallow hilly-land and deep vega soil showed (A22) a 25 per cent. increase in yield as a result of potash manuring for cacao producing naturally at the rate of 925 lb. per acre. The manure (potassium sulphate, 2½ lb. per tree) was added to trenches that had been filled with waste coconut husks. Trenching alone gave no response, but trenching plus super-phosphate (2½ lb. per tree) gave a 10 per cent. increase in yield of cacao.

NOTE.—(1) In some of the acre-block demonstration experiments in Tobago, the addition of *ammonium sulphate* increased the yields obtained by manuring with potash and phosphate, especially in shallow soils growing cacao trees having insufficient canopy to shade the soil properly.

(2) One plot of a Tobago demonstration experiment yielded at the *exceptionally high rate of 2088 lb. dry cacao per acre* in 1937-38, as a result of manuring with 2 lb. potash plus 2 lb. super-phosphate per tree. The control plot in this experiment gave a yield on that occasion of 1936 lb. per acre. In the previous year, the corresponding yields were 1174 and 1226 lb. per acre, or only about 60 per cent. as great. According to Dr. Pound's note, "both plots were given excellent husbandry and pen manure" in addition to the artificial manures.

**Special Experiments.**

(1) *A Draining Experiment at Marper.*

That draining need not necessarily in itself be an invariably successful operation in cacao cultivations in Trinidad has been demonstrated by the results of a special experiment laid down in 1930 at Marper Farm comprising heavy alluvial clay. Yields were recorded for eight successive years for cacao plots shaded to varying degrees, (33, 50 and 80 per cent. shade) by Immortel trees and drained at different distances (26, 52 and 104 feet) for each degree of shade, as compared with plots not drained. The averaged yield data (*See* Table) clearly indicate that, for an 80 per cent. degree of shade (normal for most estates in Trinidad), there is no advantage in draining such land. For diminishing degrees of shade, however, the data indicate that yields diminish as the draining distance increases. These results therefore suggest that drains and shade trees are complementary, the need for draining varying inversely with the density of the shade tree population. This important experiment furnishes concrete proof that, over a period of eight years, drains may be superfluous on cacao land carrying normal overhead shade. A similar conclusion was reached in a special demonstration experiment at River Estate, in which no response to draining and round-ridging was indicated by the first year's yield data.

A Draining Experiment on Shaded Cacao.

(Duration of experiment, 8 years).

| Draining Distance. | 80 per cent. Shade. | 50 per cent. Shade. | 33 per cent. Shade. |
|---|---|---|---|
| | (Mean yields in lb. weight dry cacao per acre). | | |
| 26 ft. | 404 | 445 | 509 |
| 52 ft. | 321 | 449 | 404 |
| 104 ft. | 508* | 278 | 280 |
| No drains | 436 | 379 | 301 |
| Averages | 417 | 388 | 374 |

* Undulating land.

(2) *A Time of Application of Manures Experiment.*

Three experiments, designed to ascertain the best time of application of manures to cacao in Trinidad, were included in Dr. Pound's programme. Two of them (Sangre Grande and Manzanilla) concern superphosphate, and one (Maracas Valley) concerns potash. In each, the times chosen for comparison were February, May and October. Results have so far been obtained for only two crops; they showed, however, that, at the first two sites (on acidic alluvial clay and on marly clay), the *October* applications of superphosphate were more productive than February or May applications. At the third site (on alluvial sand) the May application of potash manure proved the most productive. This divergence from the first result seems to be conflictory, but it should be noted that the main crop in the Maracas Valley is *three to four months earlier* than that in the Sangre Grande-Manzanilla District situated 35 miles to the east of it, and in a belt of much higher rainfall. Thus, both sets of results really coincide, and the best time for application of artificial manures would appear to be five or six months before the heaviest cropping month in Trinidad cacao fields. This application-time is coincident with the time when *young fruit is setting*, and the evidence thus supports the contention that suitable manuring at the correct period may help in affecting the survival of set fruit.

## PART II.—BIOCHEMISTRY OF CACAO.

## (1) CHANGES IN FAT AND THEOBROMINE CONTENT OF THE KERNEL OF THE CACAO BEAN DURING FERMENTATION AND DRYING.

By E. C. Humphries.

Because the fat and theobromine contents of the fermented cacao bean are of great importance to the chocolate manufacturer, it was decided to carry out an investigation into the changes which occur in fat and theobromine contents as the beans are fermenting. At the same time, changes in fat content of fresh beans allowed to sun-dry for different periods of time were also followed. It is well known that during fermentation the cotyledons lose by diffusion various water-soluble substances, so that there is an apparent increase in the fat content of the bean. Knapp (2) gives the apparent increase in fat content as about two per cent. and Doyne and Voelcker (1) as 1.5 per cent., but in the present investigation, it was found to be considerably greater than this. The object of the investigation was to determine at what stage the greatest increase in fat content occurs, and at what stage the fat content ceases to increase. The changes in theobromine content were followed at the same time.

### Experimental Procedure.

Three experiments were performed; in the first two, beans were taken from trees budded or grafted from the same parent material (Parent 1304 River Estate) and a quantity (about 40 lb.) was put to ferment in a solar fermentary of the type described by McDonald (4). Concurrently, a quantity was spread on trays and allowed to dry in the sun. Samples from both the fermenting and the sun-drying material were taken at intervals, a hundred beans selected at random constituting a sample. In the laboratory, a sub-sample was taken for moisture determination, and the remainder killed by immersion in boiling water for three minutes. The shells were removed, the kernels minced, and the powder dried in the oven at 100°C. for 24 hours. The sample was then ready for analysis. Unfortunately, in the first two experiments, circumstances did not permit of samples being taken every day.

In the third experiment, ordinary estate cacao (112 lb. in all) was put to ferment in a solar fermentary, and 200 beans were selected at random for each sample. A quantity was put to dry in the sun at the beginning of the experiment.

### Determination of Fat Content.

About 40 g. of the dry minced kernel were weighed out, and ground in a warm end-runner mill until quite liquid. The material was then transferred to a large porcelain dish, and allowed to set in a thin film, a process requiring several hours. The cocoa was then scraped off the dish and thoroughly ground in a hand mortar.

Fig. 1

Fat: Fermentation
Fat: Sundrying.
Theobromine: Fermentation.
Theobromine: Sundrying.
Water Content: Fermentation.
Water Content: Sundrying.
Experiment 1
" 2
" 3
Percentage of Original Value.
Water Content: Percentage of Fresh Weight.
Hours.
110
100
90
80
70
50
40
30
20
10
0
30
60
90
120
150
180
210
240
270
300
330

Duplicate samples of about 15 g. were placed in thimbles which were wrapped in No. 50 Whatman filter paper and placed in a Soxhlet extractor. The use of filter paper was found to be necessary in order to prevent small quantities of cocoa powder escaping from the thimble. The powder was then extracted continuously for 20 hours with petrol-ether (boiling range 50° to 60°C.). After this time, the solvent was evaporated off, and the fat dried at 105°C. and weighed.

**Determination of Theobromine.**

Theobromine was determined by the modification of Wadsworth's method described by McDonald, taking all the precautions noted in another paper of this Report.

**Experimental Results.**

Since the first two experiments were performed with similar material, they can be considered together; the figures obtained are presented separately (Table I) because the times of sampling were different in the two cases. In order that these results may be compared more closely, they have been presented graphically in Fig. 1. Here the moisture contents of the various samples of beans have also been plotted. From the results it is seen that the fat content of the fermented beans increases until about the fifth day, when it tends to remain constant. It is possible that there is a decrease in absolute fat content during fermentation, although this cannot be established without knowing the changes in the fat content of each single bean. However, there is a *relative* increase in fat content during fermentation, caused by a decrease in the absolute amount of dry matter, but the possibility that the high temperature during fermentation favours fat synthesis from carbohydrate cannot be ignored. Further evidence is needed on this point.

In the case of the air-dried beans, the fat content does not vary greatly from the initial content. On the whole, there was a very slight decrease in fat content. In the third experiment, the beans behaved in the same manner during fermentation and sun-drying as in the first two experiments. At the end of the fermentation process in the third experiment, the beans were put to sun-dry

**Table I.**

| Hours. | Fermented. | | | | Sun-Dried. | | | |
|---|---|---|---|---|---|---|---|---|
| | Fat Content. | | Theobromine Content. | | Fat Content. | | Theobromine Content. | |
| | Actual per cent. | Per cent. of Original. | Actual per cent. | Per cent. of Original. | Actual per cent. | Per cent. of Original. | Actual per cent. | Per cent. of Original. |
| | | | | Experiment I. | | | | |
| 0 | 53.06 | 100.0 | 2.74 | 100.0 | 53.06 | 100.0 | 2.74 | 100.0 |
| 42 | 53.60 | 101.0 | 2.84 | 103.6 | 52.71 | 99.3 | 2.79 | 101.8 |
| 118 | 56.24 | 105.9 | 2.47 | 90.1 | 54.10 | 102.0 | 2.97 | 108.4 |
| 162 | 55.73 | 105.1 | 2.28 | 83.2 | 54.76 | 103.2 | 3.07 | 112.0 |
| 210 | 57.87 | 109.0 | 2.09 | 76.2 | 53.04 | 100.0 | 2.86 | 104.4 |
| | | | | Experiment II. | | | | |
| 0 | 53.79 | 100.0 | 2.67 | 100.0 | 53.79 | 100.0 | 2.67 | 100.0 |
| 41 | 54.46 | 101.3 | 2.71 | 101.5 | 54.18 | 100.7 | 2.84 | 106.4 |
| 138 | 56.25 | 104.6 | 2.21 | 82.8 | 54.11 | 100.6 | 3.00 | 112.4 |
| 210 | 56.12 | 104.3 | 2.14 | 80.2 | 52.83 | 98.2 | 2.75 | 103.0 |
| 283 | 56.31 | 104.7 | 1.80 | 67.4 | 52.98 | 98.5 | 2.71 | 101.5 |
| 330 | 56.40 | 104.8 | 1.87 | 70.0 | 53.09 | 98.7 | 2.92 | 109.3 |
| | | | | Experiment III. | | | | |
| 0 | 52.62 | 100.0 | 2.88 | 100.0 | 52.90 | 100.0 | 2.64 | 100.0 |
| 24 | 52.21 | 99.2 | 2.66 | 92.4 | 53.04 | 100.2 | 2.61 | 98.9 |
| 48 | 54.32 | 103.2 | 2.57 | 89.2 | 52.65 | 99.5 | 2.66 | 100.8 |
| 72 | 55.85 | 106.1 | 2.32 | 80.6 | 52.93 | 100.0 | 2.72 | 103.0 |
| 96 | 56.26 | 106.9 | 2.23 | 77.4 | 52.53 | 99.3 | 2.73 | 103.4 |
| 120 | 56.68 | 107.6 | 2.15 | 74.6 | 52.55 | 99.3 | 2.84 | 107.6 |
| 144 | 56.47 | 107.3 | 2.11 | 73.3 | no sample | | — | — |
| 168 | 56.69 | 107.6 | 2.16 | 75.0 | 53.09 | 100.3 | 2.85 | 107.9 |
| 192 | 57.25 | 108.7 | 2.20 | 76.4 | 52.63 | 99.5 | 2.90 | 109.8 |
| 48* | 56.96 | 108.2 | 2.45 | 85.1 | — | — | — | — |
| 120* | 56.31 | 107.0 | 2.43 | 84.4 | — | — | — | — |

* Sun-drying after fermentation.

in accordance with ordinary estate practice, and samples were taken after two and after five days. Sun-drying appears to reduce the fat content somewhat, and this can be explained on the grounds that the shells of the beans are rendered more permeable by the fermentation process, so that fat diffuses outwards. The possibility that some of the fat is decomposed should also be considered. The total loss of fat is not great, and the net apparent increase in fat content after fermentation is of the order of seven per cent. (7%) on the original content, or about four per cent. (4%) on an oven-dry basis.

A loss of theobromine occurs during fermentation, as found by previous investigators (3). This loss is approximately linear at first, but the indications are that, after some time, the theobromine tends to reach a steady value (compare the value after 283 hours with the value after 330 hours in Experiment 2). Fermentation is rarely carried on longer than 7 days in estate practice, and up to this point there is a steady decrease in theobromine content. In contrast to the fat content, the absolute *decrease* in theobromine content is masked by the fact that the absolute dry matter content is all the time decreasing. When the beans are sun-dried after fermentation, there is a slight increase in the theobromine content (Table I), caused apparently by loss of volatile constituents during drying. The apparent decrease in dry-weight as reflected by the increase in fat content is insufficient to account for the increase in theobromine. It is unlikely there would be a decrease in absolute dry-weight caused by loss of solids during drying.

In all three experiments, the theobromine content increased in the beans that were subjected only to sun-drying. Here again, it is unlikely that solids would be lost during this process. There are therefore two possible explanations: (*a*) volatile constituents are gradually lost during the drying process, and (*b*) drying breaks up the theobromine-tannin complex which according to some authors is present in the bean, and thus increases the free theobromine content. This latter explanation seems improbable for two reasons. Firstly, during fermentation, the kernel is permeated with an acidic liquid of about pH 4.0 which would probably liberate all the theobromine in a free state, and secondly, in the estimation of theobromine, the fat-free cocoa powder is mixed with water and magnesium oxide with the object of decomposing any theobromine compound. It seems, therefore, that the loss of volatile constituents from the bean during sun-drying is quite considerable.

The beneficial effects of fermentation noted in this paper, namely the increase in fat content and the decrease in theobromine content, are apparently diminished during the sun-drying process which the beans undergo after fermentation.

### Summary.

(1) The changes in fat and theobromine contents of the cacao bean during fermentation and sun-drying were investigated.

(2) The fat content of the fermented beans increased until about the fifth day. The amount of the increase was about four per cent. (4%) on an oven-dry basis. There was a slight decrease in fat content during sun-drying after fermentation.

(3) The fat content of beans which were sun-dried only did not alter appreciably.

(4) There was a loss of theobromine during fermentation. The theobromine content tends to reach a constant value after 12 days, but this is an excessive time for fermentation. When the beans were sun-dried after fermentation, the theobromine content increased slightly. The theobromine content increased in the beans which were subjected only to sun-drying.

### Acknowledgments.

The author is indebted to Mr. R. O'Connor, Manager of River Estate, for providing facilities for collecting pods and carrying out a fermentation, and to Mr. J. Carrington for performing the analytical determinations.

### References.

(1) Doyne, H. C. and Voelcker, O. J.—*Trop. Agric.*, 1939, XVI, p. 76.

(2) Knapp, A. W.—Cacao Fermentation, p. 101. London, 1937.

(3) Knapp, A. W. and Wadsworth, R. V.—*Journ. Soc. Chem. Indus.*, 1924, 43, pp. 124-126 T.

(4) McDonald, J. A.—*Fifth Annual Rept. Cacao Res.*, 1935, pp. 48-55.

## (2) A NEW METHOD FOR ESTIMATING TOTAL ALKALOIDS IN CACAO.

By E. C. Humphries.

While the estimation of theobromine by extraction with tetrachlorethane in a Soxhlet could perhaps be regarded as satisfactory for routine analysis, it is subject to several errors which have been pointed out (1). It seemed necessary therefore to search for a method which would give more concordant results, and which would include the estimation of the other alkaloid present in cacao, namely caffeine. So far, the only reliable method available for the purpose is Dekker's method. This entails so much manipulation that errors may arise, and moreover there is the great danger that extraction might be incomplete. Dekker used chloroform as the solvent for the alkaloids and this lends itself readily to a continuous extraction method. The solubility of theobromine in chloroform is given by Wadsworth (3) as 0.06 g. per 100 cc. at 15.5°C., and 0.07 g. per 100 cc. at the boiling point, so that, given a sufficiently long extraction, chloroform

should extract all the theobromine from a sample of cocoa powder. In practice, it was found that such a method gave reproducible results. The method finally adopted was as follows :—

Five grams of fat-free cocoa powder are mixed with 2.5 grams of freshly-calcined magnesia, and about 10 cc. of water added drop by drop from a burette until all the particles are wetted. The powder is then heated in a porcelain dish on a water-bath and partially dried. This process takes about half-an-hour. It is then well mixed and wrapped in a No. 41 Whatman filter paper, placed in an extraction thimble, and extracted in a Soxhlet apparatus with chloroform for 20 hours. The solvent is evaporated off, and the residue dried at 100°C. This residue contains, besides the alkaloids, some fatty material which forms the subject of another paper in this report. The residue is allowed to stand overnight in 100 cc. of petrol-ether which removes the fat, and the solid matter now represents the total alkaloids, which can be collected in a weighed Gooch crucible, dried at 100°C. and weighed. The dried residue is then treated with 100 cc. benzene and allowed to stand for a few hours. This removes the caffeine. The solid residue is collected in an asbestos lined Gooch crucible and weighed to find the amount of theobromine. The weight of caffeine is estimated by difference.

Some results, obtained at different times on the same sample of good Forastero cacao, are given below :—

| Total Alkaloids (per cent.) | Theobromine (per cent.) | Caffeine (per cent.) | Time of Extraction (hours). |
|---|---|---|---|
| 2.69 | 2.43 | 0.26 | 20 |
| 2.72 | 2.53 | 0.19 | 20 |
| 2.71 | 2.50 | 0.21 | 20 |
| 2.70 | 2.46 | 0.24 | 20 |
| 2.75 | 2.54 | 0.21 | 20 |
| 2.65 | 2.36 | 0.29 | 30 |
| 2.67 | 2.41 | 0.26 | 30 |

The agreement between samples is satisfactory, and it is evident that a 20-hours extraction is sufficient to extract all the alkaloids from the powder.

**Alkaloid Content of Different Varieties of Cacao.**

The alkaloid content of different varieties of beans was next determined by this method. Trinidad Forastero cacao being a hybrid population can only be divided into classes arbitrarily, and two types were selected which fall towards the extremes of the quality range. To represent the lower end of the range, smooth, almost spherical pods were used, of the type generally called " Calabacillo ". For the upper end, long, yellow, furrowed pods were used, obtained from budded trees at River Estate (Parent 1304), and these for convenience are called " Good Forastero ". For comparison with the two Forastero types, two strains of Criollo were available, which are probably of Nicaraguan origin and distinct from the parental stock of Trinidad cacao.

The following are the results obtained :—

| | Alkaloids (per cent.) | Theobromine (per cent.) | Caffeine (per cent.) |
|---|---|---|---|
| " *Criollo* " Variety $V_0$ | 3.05 | 2.15 | 0.90 |
| | 3.19 | 2.25 | 0.96 |
| | 3.36 | 2.31 | 1.05 |
| Variety 48 | 1.73 | 1.23 | 0.51 |
| | 2.04 | 1.05 | 0.99 |
| " *Good Forastero* " (mean of above results) | 2.70 | 2.46 | 0.24 |
| " *Calabacillo* " | 3.63 | 2.90 | 0.73 |
| | 3.59 | 2.85 | 0.74 |
| | 3.57 | 2.86 | 0.71 |

Comparisons between results obtained by different workers is complicated by the wide genetical variation of cacao, and by varying interpretations put upon such terms as " Forastero " and " Calabacillo ". It is, however, of interest to note that the figures obtained for the theobromine content of the different varieties agree well with these previously published by Wadsworth (4). Thus, the Criollo cacao has the smallest theobromine content, good Forastero is intermediate, and Calabacillo has the highest content. The figures published by Kaden (2) show a reverse order.

The Criollo varieties have a comparatively high percentage of caffeine, while the good Forastero variety contains very little caffeine. Calabacillo contains the highest percentage of total alkaloids.

**Summary.**

A new method for the determination of theobromine and caffeine in cacao is described.

Analyses of the alkaloid content of beans of different varieties of *Theobroma cacao* are presented.

**References.**

(1) Humphries, E. C.—*Eighth Annual Rept. Cacao Res.*, 1938, p. 38.

(2) Kaden, O. F.—*Tropenpflanzer*, 1935, 38, No. 9.

(3) Wadsworth, R. V.—*Analyst*, 1920, 45, p. 133.

(4) Wadsworth, R. V.—*Analyst*, 1922, 47, p. 152.

## (3) A CRITICAL INVESTIGATION OF THE TETRACHLORETHANE EXTRACTION METHOD FOR DETERMINING THEOBROMINE IN CACAO.

By E. C. Humphries.

It was found during routine determinations of theobromine by extraction with tetrachlorethane in a Soxhlet apparatus (2) that, while fairly good agreement between duplicates could be obtained, the results were not always reproducible. An attempt was therefore made to discover the errors of the method, and its advantages over the method of Wadsworth (1).

The first fact that became evident was that, under the conditions originally laid down by McDonald (2), a long extraction is necessary (up to 20 hours) in order to remove most of the theobromine from the fat-free powder. The long extraction, however, has the disadvantage of removing colouring matter and other impurities from the powder, with consequent adulteration of the theobromine sample. The efficiency of the extraction was greatly increased by reducing the amount of fat-free powder from 8 g. to 5 g. This provided sufficient theobromine for weighing, and at the same time, it was found that the period of extraction could be reduced to 12 hours.

In preliminary experiment, the original Wadsworth method was compared with McDonald's modification, and it was found that the former was quicker than the latter, but not in all cases did the Wadsworth method give a complete extraction. By estimating the amount of theobromine obtained by successive extractions by the Wadsworth method, it was found that no theobromine was obtained in the third boiling, although the extraction of the theobromine from the powder was known to be incomplete. A typical case is given in the table.

| Theobromine Extraction. | Per cent. of Total Extracted. | |
|---|---|---|
| | A | B |
| 1st Extraction ... ... | 90.19 | 94.66 |
| 2nd do. ... ... | 9.81 | 5.34 |
| 3rd do. ... ... | — | — |

The incomplete extraction may perhaps be accounted for by the accumulation of filter papers from successive extractions, but experiments in which the cocoa powder was wrapped in a cloth bag and extracted with four successive quantities of solvent proved unsatisfactory. In most cases where the original Wadsworth method and McDonald's modification were compared, it was found that the latter gave a greater yield of theobromine. It was thought that this result might be due to the presence of greater amounts of impurities in the Soxhlet extraction, but estimation of ash and residue after dissolving the theobromine in hot water showed that this was not the case. The following are some results obtained by the two methods.

| | Per cent. Theobromine in fat-free powder. | |
|---|---|---|
| | Wadsworth. | McDonald. |
| Sample 1 (Forastero) | 2.30 | 2.73 |
| | 2.23 | 2.80 |
| | 2.21 | 2.90 |
| | 2.23 | 2.81 |
| Sample 2 (Criollo) | 1.47 | 1.84 |
| | 1.34 | 1.74 |
| | 1.41 | 1.79 |

Note.—In all cases, corrections for impurities in the theobromine residues have been made.

McDonald's method gave consistently higher values for theobromine. This may be explained by any of the following reasons:—

(1) More complete extraction by McDonald's method.

(2) Extraction of substances other than theobromine by McDonald's method, such substances being either unextracted or destroyed by the Wadsworth method where the solvent is kept at boiling point.

(3) Destruction of theobromine in the Wadsworth method by the boiling solvent.

As a result of the foregoing experiments, it was decided that McDonald's modification is preferable to the original Wadsworth method in two respects, namely,

(1) the extraction of theobromine is more complete,

(2) the method is more straightforward and less manipulation is necessary, although the time taken is longer.

Experience with the method, however, showed that it is liable to certain errors, and experiments were accordingly carried out in an attempt to trace these errors, so that the method could be relied upon to give reproducible results. It was suspected that variations in results might be caused by slight differences in the mode of preparation of the material. According to Wadsworth (*loc cit.*, p. 36), weak alkali is necessary for the decomposition of the theobromine compound present in the cacao bean, and some water must be present in the solvent for complete extraction. As a routine practice, therefore, the cocoa powder is mixed with magnesia and a certain

amount of water, and a part of this water is subsequently removed by evaporation. The amount of water left in the powder can only be judged by appearance, and this seems likely to be a source of error. In order to find out what parts the magnesia and water played in regard to possible error, a series of experiments were performed.

In several experiments the fat-free cocoa powder was mixed with the usual quantity of magnesia and then thoroughly mixed with about 10 cc. of water which was added, drop by drop, from a burette. This mixture was extracted without any of the water being removed. The average result was 2.02 per cent. theobromine, whereas the theobromine content of the powder was known to be approximately 2.65 per cent. The excess of water in some way interfered with the extraction. A number of experiments were then performed, in which the cocoa powder and magnesia were triturated with water, the mixture dried in the oven at 100°C. for 24 hours, and the dry powder extracted with tetrachlorethane. The results were very low, averaging 1.3 per cent. The expedient of extracting the oven-dried material with tetrachlorethane to which 1 cc. of water was added did not appreciably increase the quantity extracted. It was concluded that the amount of water remaining in the mixture is of some importance, hence great care must be taken to ensure that the powder is prepared in exactly the same way in all determinations.

During the progress of these experiments, an important fact emerged, namely, that just as big a yield of theobromine is obtained by extracting the wetted cocoa powder with tetrachlorethane as is obtained when the powder is mixed with magnesia. This result was thought to depend on the fact that the tetrachlorethane used (technical quality) contains free acid which serves to split the theobromine-complex as does magnesia.

**Summary.**

The relative merits of the Wadsworth and McDonald methods for estimation of theobromine were investigated. The latter method was found to be more satisfactory. Magnesia is not essential for mixing with the fat-free powder when tetrachlorethane is used as solvent.

**References.**

(1) Wadsworth, R. V.—*Analyst*, 1921, 46, pp. 32-37.
(2) McDonald, J. A.—*Sixth Annual Rept. Cacao Res.*, 1936, pp. 43-44.

## (4) A NOTE ON THE ESTIMATION OF FAT IN CACAO.

By E. C. Humphries.

During an investigation of a method for the determination of total alkaloids in cacao, it was found that a further small quantity of fat could be extracted from what was normally taken to be fat-free powder. This "fat-free" powder was obtained by extracting the kernel for 20 hours with petroleum ether (boiling range, 50°-60°C) after the kernel had been subjected to a special grinding process in a mechanical mortar.

In the method for the extraction of the alkaloids, it is necessary to mix the fat-free powder with freshly ignited magnesium oxide and water. On extracting this mixture with chloroform, not only are the alkaloids removed, but also some fatty material. This is readily soluble in cold petrol-ether the xanthine bases remaining undissolved.

It was not claimed that the petrol-ether extraction removed all the fat from the cacao, but it was surprising to find such a large quantity extracted by the chloroform. The residue obtained by evaporation of the chloroform extract represented the extra fat together with the alkaloids (theobromine and caffeine). On treating this residue with petrol-ether in the cold, the fat dissolved, leaving the alkaloids. The difference in weight between the residue from the chloroform extraction and the weight of alkaloids gives the weight of fat which should be added to the fat obtained by petrol-ether extraction.

Three portions of cacao from the same sample were extracted with petrol-ether for 20 hours in the usual way, mixed with magnesia in the manner previously described (1), and then further extracted with chloroform for 20 hours, and the extra fat in each case determined by subtracting the weight of total alkaloids. By this procedure, it was found that the values for fat content could be brought into much better agreement (Table I).

**Table I.**

| | Fat Content Determined by Petrol-ether Extraction (per cent.) | Fat by Chloroform Extraction (per cent.) | Total Fat (Corrected) (per cent.) |
|---|---|---|---|
| 1 | 52.74 | 2.08 | 54.82 |
| 2 | 52.14 | 2.73 | 54.87 |
| 3 | 53.35 | 1.60 | 54.95 |

It is generally considered extremely difficult to remove the last traces of fat from plant material by extraction with ether or petrol-ether (2), the degree of efficiency depending to a certain extent on the state of sub-division of the material. Since in the present investigation the material

had been thoroughly ground in an end-runner mill, it was considered that particle size was not the limiting factor.

In order to find if prolonged extraction of the cocoa powder with petrol-ether would ultimately remove as much fat as the combined extractions with petrol-ether and chloroform, some "fat-free" powder, obtained by 20 hours' extraction with petrol-ether was extracted for a further 20 hours. It was found that an additional quantity of fat was extracted (amounting to about 1 per cent.) *i.e.* rather less than obtained by the chloroform method. It thus appears that either (i) the treatment of the powder with magnesium oxide facilitates the extraction of residual fat or (ii) the residual fat is more readily soluble in chloroform. The latter alternative seems likely, since the fat fraction extracted by chloroform appeared different from ordinary cacao fat and tended to darken on keeping.

**References.**

(1) Humphries, E. C.—*Eighth Annual Rept. Cacao., Res.*, 1938, p. 37.
(2) Lockwood, H. C.—*Analyst*, 1938, 63, p. 705.

## (5) THE BIOCHEMISTRY OF THE CACAO BEAN; PRESENT STATE OF KNOWLEDGE.

By H. F. Birch and E. C. Humphries.

The following is a brief survey of investigations performed during the past ten years on the biochemistry of cacao; its scope shows that outstanding results have been obtained only where the prepared bean has been investigated. Notable in this respect has been the discovery of the high vitamin D content of the cacao shell.

With regard to investigations designed to follow the course of the changes in certain constituents of the bean during the course of fermentation and ripening, the results obtained have shown in some cases a number of discrepancies which are particularly apparent in the case of tannins. It cannot be said therefore that satisfactory progress has been made in our knowledge of certain constituent changes in the bean which may account for the superiority of fermented over unfermented cacao. The lack of satisfactory analytical methods in some cases, and the limited number of workers in a position to investigate such changes, must be considered as factors contributing to the slow development in this field.

### (1) The Tannins of the Cacao Bean.

The first specific study of the tannins of cacao was published by Jensen (14), who determined the water-soluble tannin content of fully roasted cacao nib, and obtained values ranging from 5.2 to 6.5 per cent. Adam (2) investigated both the catechin and the tannin contents of fermented and unfermented cacao. He found that no catechin was present in the fermented beans whereas changes in the tannin content as a result of fermentation appeared to be less drastic. Similar investigations undertaken by McDonald (29), showed that tannin substances are present in considerable quantity both in the shell and in the kernel of the cacao bean, but no definite correlation could be established between the tannin content of different botanical types. Contrary to Adam's results, McDonald found a greater content of tannin in fermented cacao beans as compared with unfermented beans of the same type.

Duthie (8) modified McDonald's method of tannin analysis to include a separate determination of what was considered to be catechin and similar phenolic substances. He found little difference either in the tannin contents of fresh Criollo and Forastero beans or in the contents of other polyphenolic substances in these beans. A decrease in the amount of certain phenolic substances present in the bean was observed during fermentation. The tannin content, in agreement with Adam's results, was found to remain sensibly constant.

The catechin-like substance present in fresh cacao-beans of Criollo, Forastero-Criollo hybrids and Forastero types as well as in cacao beans grown in Guayaquil, Costa Rica and Bahia, was investigated by Adam, Hardy and Nierenstein (3) and identified by Nierenstein as l-acacatechin. Subsequent work by Freundenberg, Cox and Braun (10), who isolated the same compound from cacao from Trinidad, Puerto Rico and Sumatra showed the catechin substance to be l-epicatechin, a correction which Nierenstein has accepted.

Recently a method has been devised by Lilienfeld-Toal (28) to follow the course of cacao fermentation by means of permanganate titration. The method gives a measure of the changes in total oxidisable water-soluble compounds during fermentation, but it provides no information regarding variation in any specific constituents.

### (2) The Sterols and Vitamins of Cacao.

Since Labbé and co-workers (19, 21) isolated $\alpha$-and $\beta$-sterols from the germ, nib and shell of cacao, and found that these compounds (particularly the $\beta$-sterol, of which cacao shell is the richest source) possessed anti-rachitic properties after irradiation with ultra violet light, Knapp and Coward (15, 16) have shown cacao kernel to contain an amount of Vitamin D equal to 1 International unit, and cacao shell to contain an amount equal to 28 International units per gram. Achcenich (1)

has recorded a Vitamin D content of 125 units in cacao shell. The presence of important amounts of a vitasterol similar to Vitamin A has been demonstrated by Labbé and co-workers (24, 12) in cacao shell, and a small quantity in the kernel in which a trace of Vitamin A was also found. An assay of the Vitamin B content of cacao by the same workers (22, 23) showed that the pericarp of fresh and dried beans and shells do not contain any appreciable amount of Vitamin B, but that it is present in commercial cacao germs. The presence of antineuritic Vitamin B in cacao beans has also been proved by Sasaki and Wakayama (35), and of Vitamin E in cacao embryo by Labbé (25).

### (3) Colouring Matters.

With regard to the colouring matters present in cacao, Adam (2) has expressed the opinion that the red and brown colouring matters of fermented and sundried cacao are complex alteration products of the catechin and tannin originally present in the bean. Fincke (9) has distinguished between two pigments, namely, *cacao brown*, which is produced gradually from a colourless parent substance during fermentation, and *cacao red*, which occurs in varying amounts in fresh seeds. Fincke has also suggested that both cacao red and cacao brown are tannin substances, but offers no proof in support of this view. A microscopic examination of fresh coloured beans by Knapp (17) revealed a purple pigment against a background of colourless cells. In a subsequent communication, the same author (18) has suggested that this purple pigment hitherto known as cacao red should be termed *cacao purple*, and that, during fermentation, this pigment, as well as colourless cacao catechin and tannin, are changed wholly or in part to brown substances.

Investigations by Steinmann (36) on the colouring matter of cacao beans led him to advance the theory that the formation of free cacao purple is a photochemical process. Knapp (17), however, was unable to confirm Steinmann's conclusion. The purple pigment has recently been shown by Robinson to be cyanidin 3-glycoside, but no attempt has yet been made to follow the course of changes in this pigment during fermentation.

### (4) Lecithin in Cacao.

Whether cacao contains lecithin is doubtful. Rewald and Christlieb (33,34) found in a variety of beans an average phosphatide content of 0.1 per cent., and subsequent experiments led them to believe that the phosphatide is not distributed in the fat, but, as in other foods, is combined with protein substances. The determination of the choline content of several commercial cacao preparations has been carried out by Nottbohm and Mayer (30). Although choline is the chief constituent of egg-yolk lecithin, the presence of choline in cacao does not necessarily indicate lecithin, and in a later communication, the same authors (31) express doubt as to whether lecithin actually occurs in cacao. They suggest that phosphatides other than lecithin may be present, or that choline exists in another kind of combination.

### (5) Cacao Fat.

Cacao fat is one of the chief components of the cacao bean, and is an important by-product in chocolate manufacture. It is a mixture of glycerides, the exact proportions of which have been given variously by different authors, but Lewkowitsch (27) has reconciled the results of Amberger and Bauch (4) with those of Lea (26), making the approximate composition :—

| | | |
|---|---|---|
| palmito-oleo-stearin | .... | 55 per cent. |
| oleodistearin .... | .... | 25 do. |
| palmito-diolein | .... | 20 do. |

The glycerides appear to be in the form of homogeneous mixed crystals, which can apparently exist in two modifications, a stable $\alpha$-form and metastable $\beta$-form (32).

Little is known about the variation in composition of cacao butter from different kinds of cacao. That such differences exist is indicated by variations in the temperature range over which solidification and melting take place. Until more is known about the inter-relationships of the botanical varieties it would probably be of very little consequence to investigate this aspect unless it could be shown that some kinds of cacao butter are of superior quality to others.

There is quite a wide range in the percentage fat content of the cacao kernel, and this, besides being a varietal difference, has also been shown to be a seasonal effect (7). The relative fat content of the bean increases during fermentation (7, 13, 17), but it is not known with certainty whether the composition of the fat alters during the fermentation process. Again it is not known whether the composition of the fat alters during the later stages of ripening. The stage at which the pod is picked may be important as regards the composition of the fat. No work has ever been attempted on the development of the fat in the bean.

Various authors have failed to demonstrate the presence of *lipase* in the cacao bean. Ciferri (6) claims to have found it in the dried unfermented bean, but recent attempts in this laboratory to detect it in fresh, dried and fermented beans were unsuccessful. There can be no doubt that lipase is present, since fat is the chief food reserve in the cotyledons, but its action appears to be inhibited by some substance or substances.

The germ itself contains from three to four per cent. of an oil which has recently been investigated (5). This oil contains about 12 per cent. of unsaponifiable matter, from which sterols have been isolated.

### (6) The Alkaloids of Cacao.

The bitter xanthine bases, theobromine and caffeine, are important in connection with cacao quality. These closely allied compounds occur in different proportions in different varieties, Criollo cacao usually containing a relatively high

proportion of caffeine. These alkaloids are formed at a relatively late stage in the ripening of the bean (unpublished results, this laboratory), but nothing is known about the effect of external conditions in altering the relative proportions of the two substances; in other words, it is not known how far the proportions are a genetic factor. Both theobromine and caffeine appear to be combined with a tannin substance (17), but little is known about the nature of the combination. Some authors consider that alkaloids are abnormal products of nitrogen metabolism (11), whereas others consider they comprise nitrogen reserves needed for protein synthesis (37). It is possible that, under conditions favourable for protein synthesis, the amount of xanthine bases would diminish.

### (7) Quality in Cacao.

The foregoing survey of the present state of knowledge of the biochemistry of the cacao-bean indicates that many isolated facts have been established with regard to both fresh and fermented beans, but we are still very far from being able to state what biochemical factors are important in deciding *quality*. Progress in this direction is hampered by the fact that the manufacturers themselves are unable to define quality in simple terms. Even when this has been done, their definition would need to be translated into terms of fresh bean composition, taking into account the complex changes which occur during fermentation, sun-drying and roasting. Nevertheless, if the manufacturers could give a clear statement of the desirable qualities in cacao (apart from such criteria as large rounded beans, absence of mould, &c.), some progress might be made in deciding what factors are of importance in deciding quality.

There is still one method of approach available; some varieties of cacao (notably Criollo and Criollo-Forastero hybrids) find more favour with the manufactureer than the coarser varieties (such as Calabacillo), and by a comparison of the beans, it ought to be possible to obtain some idea of the important differences between the three main types of cacao. So far, a mere comparison of the mature beans has failed to reveal any striking difference in the main chemical components, but it is likely that, if the developmental history of each variety were followed, bigger differences between them might be disclosed. Evidently the problem of quality is very involved, and its elucidation will necessitate a complete understanding of the biochemistry of the cacao bean during development and maturation, as well as during fermentation and sun-drying.

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